METABOLIC EFFECTS OF EXERCISE IN ADOLESCENT OBESITY

G.J. van der Heijden

Metabolic Effects of Exercise in Adolescent Obesity

Gert-Jan van der Heijden

Metabolic Effects of Exercise in Adolescent Obesity

1. Training van het cardiorespiratoire uithoudingsvermogen of krachttraining leidt tot een verbetering van parameters van het metabool syndroom, ook zonder het optreden van gewichtsverlies. (dit proefschrift)
2. Cardiorespiratoire training kan mogelijk niet-alcoholische leververvetting tegengaan ook zonder het optreden van gewichtsverlies. (dit proefschrift)
3. Perifere en hepatische insulinesensitviteit zijn twee verschillende mechanismen voor het handhaven van normoglycemie. Een training in de toename van lichamelijke aktiviteit resulteert in een toegenomen insulinesensitiviteit op beide niveaus. (dit proefschrift)
4. In tegenstelling tot adolescenten met een normaal gewicht neemt de vetoxidatie gemeten over 24 uur in obese adolescenten niet toe als gevolg van cardiorespiratoire training. Dit duidt op een metabole inflexibiliteit in deze populatie. (dit proefschrift)
5. Een normale BMI bij adolescente meisjes sluit het bestaan van aan obesitas gerelateerde metabole risicofactoren niet uit. (dit proefschrift)
6. Het gegeven dat chirurgie aan de maag, met als doel de voedselinname bij kinderen met ernstige obesitas te verminderen, een geaccepteerde methode is, betekent dat bij lange na niet genoeg aandacht bestaat voor de preventie van obesitas.
7. Als ouder ben je verantwoordelijk voor de gezondheid van je kind. Ernstige, niet syndromale obesitas op de kinderleeftijd kan worden opgevat als een vorm van kindermishandeling.
8. Portugees spreekwoord: "De pequenino se torce o pepino" "Men kan de komkommer vervormen wanneer hij jong is." Analoog hieraan moet de preventie van de metabole complicaties van obesitas gedurende de jeugd beginnen.
9. Gymnastiekles moet kinderen enthousiasmeren voor sport en bewegen, en is daarmee één van de belangrijkste schoolvakken. Het zou iedere dag minimaal één uur gegeven moeten worden.
10. Zorg is een basisvoorziening en mag niet aan de vrije marktwerking worden overgelaten.
11. "Poverty is the world's worst human rights crisis." (The Unheard Truth, Poverty and Human Rights, Irene Khan, secretaris-generaal van Amnesty International)

Gert-Jan van der Heijden, 10 December 2009

The studies presented in this thesis were performed at the USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine®, and the General Clinical Research Center at Texas Children's Hospital®, Houston, Texas, United States of America.

This work is a publication of the U.S. Department of Agriculture/Agricultural Research Service, Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas. The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement from the U.S. government.

The studies were supported by NICHD RO1 HD044609; Baylor General Clinical Research Center Grant MO1-RR-00188-34 and USDA CRIS 6250-51000-046.

The research presented in this thesis was performed within the framework of the Graduate School for Drug Exploration (GUIDE) (RuG)

Metabolic Effects of Exercise in Adolescent Obesity / Gert-Jan van der Heijden, Thesis, Rijksuniversiteit Groningen, The Netherlands

Cover design: M.A.B. van der Vlist
Lay-out: Legatron Electronic Publishing, Rotterdam, The Netherlands
Printed by: Ipskamp Drukkers BV, Enschede, The Netherlands

Financial support for printing of this thesis was received from GUIDE, AstraZeneca BV and Nestlé Nutrition BV

ISBN/EAN: 978-90-367-4191-0

Table of Contents

cmb.

Rijksuniversiteit Groningen

Metabolic Effects of Exercise in Adolescent Obesity

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
woensdag 3 februari 2010
om 14:45 uur

door

Gert-Jan van der Heijden

geboren op 7 juli 1980
te Rotterdam

Promotores : Prof. dr. P.J.J. Sauer
Prof. dr. A.L. Sunehag

Beoordelingscommissie : Prof. dr. D.M. Bier
Prof. dr. H.A. Delemarre–van de Waal
Prof. dr. R.P. Stolk

Chapter 1

General introduction and outline of the thesis

The increasing prevalence of overweight and obesity in children and adolescents and the corresponding rise in its co-morbidity, e.g. type 2 diabetes and fatty liver disease, is a serious public health concern. Interventions are urgently needed to prevent and delay the development of this morbidity. This thesis focuses on controlled exercise programs as a tool to counteract the metabolic effects of obesity in the adolescent population.

Epidemiology

Over the past decades, the prevalence of obesity and obesity related illnesses has increased dramatically in children and adolescents. Whereas in 1966-1979, 5% of American adolescents was obese (1) i.e. body mass index > 95th percentile for age (2), in 2003-2004 a staggering 17% reached the criteria of obesity (3). The highest prevalence is observed in Hispanic children and adolescents (3). The same trend is observed worldwide (4). Childhood obesity increased in virtually all industrialized countries such as the Netherlands, as well as in several lower-income countries (4,5).

Obesity and disease

In adults, obesity is associated with significant health risks such as insulin resistance, type 2 diabetes, increased blood lipids, hypertension, coronary heart diseases and certain types of cancer (6). Overweight in adolescence is associated with adult overweight and related cardiovascular risk factors (7). Currently, obesity associated metabolic abnormalities such as increased incidence of type 2 diabetes (8) can already be observed in children and adolescents. It has been reported that 15-30% of all newly diagnosed diabetic adolescents have obesity related type 2 diabetes (9,10), 30-50% of obese children have the metabolic syndrome (11), and 30-40% of obese children have non alcoholic fatty liver disease (12,13).

Factors leading to obesity

Many factors may contribute to the development of obesity in children and adolescents, e.g. parental obesity (including both genetic and environmental components), total energy intake, relative fat intake and reduced energy expenditure. A very important factor, and the focus of this thesis, is reduced (habitual) physical activity and increased sedentary behaviour (14,15). Troiano et al. (16) recently reported that only 8% of American adolescents (12-19 y) obtained the recommended 60 min per day of physical activity.

In short, weight gain is the result of an imbalance between energy intake and energy expenditure. Excess energy intake will be deposited as excess body fat, and excess body fat is the major cause of obesity related morbidity (17).

Insulin sensitivity and body fat composition

Our previous studies demonstrated that apparently healthy, obese adolescents already had 50% lower insulin sensitivity than their non-obese counterparts and they secreted twice as much insulin to maintain normoglycemia (18). The increased demands on the pancreatic ß-cells might subsequently lead to type 2 diabetes.

The mechanism behind the development of reduced insulin sensitivity in obese children, adolescents and adults is not fully understood. Total body fat percentage is strongly associated with reduced insulin sensitivity. Interestingly, fat stored in specific body fat depots (abdominal fat and fat within the hepatic and muscle cell) is thought to play a metabolically important role (17). Notably, hepatic fat deposition in itself is a serious disorder that can progress to liver inflammation, fibrosis and cirrhosis (12).

In children and adolescents, obesity and over weight are most often defined by age related percentiles of Body Mass Index (BMI) [weight in kg/(height in m)^2] (2). BMI might, however, not be an optimal marker of leanness/obesity (19). Adolescents with normal BMI can have high fat mass (19,20). Consequently, using BMI alone for screening, adolescents with normal BMI but high body fat percent (≥ 27%) (21) would inappropriately be classified as lean and thus, at low risk of metabolic disorders

Interventions to prevent and delay obesity and its co-morbidity

Strategies to prevent and delay obesity and its co-morbidity in children and adolescents are much needed.

Various lifestyle interventions have been shown to reduce obesity and its co-morbidities. However, studies combining lifestyle advice, diet and exercise programs make it difficult to determine which intervention is most efficient and best accepted by this population. Although weigh loss improves metabolic disturbances it is difficult to achieve compliance with energy reduced diets, often resulting in weight regain. Thus, this approach is seldom successful. However, will a moderate exercise program that sedentary obese adolescent can comply with be efficacious?

Several studies in adults have demonstrated that regular moderate exercise improves insulin sensitivity and reduces the risk for cardiovascular disease, type 2 diabetes and certain types of cancer (22-24). In addition, a limited number of studies have

demonstrated that exercise has positive metabolic effects in children and adolescents (25-28). However, many of these studies incorporate different combinations of various more or less controlled interventions including dietary advice, making it difficult to determine the effect of exercise itself.

Two major types of exercise exist: aerobic (running, biking, swimming) and resistance (strength) exercise. Positive outcomes have been found for both types of exercise, but it is currently not well studied how the metabolic effects of a moderate aerobic or resistance exercise program compare and if there is an optimal exercise intervention.

The objective of this thesis is to determine in depth, the metabolic effects of moderate intensity aerobic and resistance exercise programs without dietary intervention or weight loss in sedentary lean and obese adolescents.

Outline of the thesis

The first aim of this thesis is to determine the effect of a moderate aerobic exercise program on body composition, insulin sensitivity, glucose and lipid metabolism and energy expenditure in lean and obese sedentary adolescents

The following questions are addressed:

- What is the effect of aerobic exercise on body composition and body fat distribution (abdominal, hepatic and intramyocellular) and is body fat distribution and insulin resistance correlated in lean and obese adolescents? (**Chapter 2**)
- What is the effect of aerobic exercise on peripheral and hepatic insulin sensitivity and glucose and lipid metabolism in lean and obese adolescents? (**Chapter 3**)
- What is the effect of an aerobic exercise program on total 24 h energy expenditure and its components, basal, sleep and awake sedentary energy expenditure and substrate oxidation in lean and obese adolescents? (**Chapter 4**)

The second aim of this thesis is to determine the metabolic effects of a moderate resistance (strength) exercise program in obese adolescents and to compare its effects with the results of the aerobic exercise program.

The following questions are addressed:

- What is the effect of resistance exercise on body composition and body fat distribution (abdominal, hepatic and intramyocellular)? (**Chapter 5**)
- What is the effect of resistance exercise on peripheral and hepatic insulin sensitivity and glucose and lipid metabolism? (**Chapter 5**)

- How do the metabolic effects of resistance exercise compare with those of aerobic exercise? Is there an optimal exercise intervention for obese adolescents? (**Chapter 5**)

Body mass index (BMI) does not provide information about body fat percentage (%) and distribution. Adolescents (girls in particular) can have normal BMI but high body fat %. These adolescents might be at risk for obesity related disorders. The metabolic impact of normal BMI but a high body fat % is currently unknown. The third aim of this thesis is to determine whether adolescent girls with normal BMI but high body fat % have increased abdominal fat content and exhibit risk factors for obesity-related co-morbidity. The following question is addressed:

- Are adolescent girls with normal BMI but high total body % at risk for obesity related co-morbidity? (**Chapter 6**)

Finally, in Chapter 7 we summarize and discuss the main findings of the thesis and provide future perspectives with respect to the role of exercise in preventing and decreasing obesity and its co-morbidity.

References

1. National Center for Health Statistics: website. Prevalence of obesity (1999-2000).
2. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. *Adv Data* 2000:1-27.
3. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 2006;295:1549-55.
4. Wang Y, Lobstein T. Worldwide trends in childhood overweight and obesity. *Int J Pediatr Obes* 2006;1:11-25.
5. van den Hurk K, van Dommelen P, van Buuren S, Verkerk PH, Hirasing RA. Prevalence of overweight and obesity in the Netherlands in 2003 compared to 1980 and 1997. *Arch Dis Child* 2007;92:992-5.
6. Poirier P, Giles TD, Bray GA, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 2006;113:898-918.
7. Srinivasan SR, Bao W, Wattigney WA, Berenson GS. Adolescent overweight is associated with adult overweight and related multiple cardiovascular risk factors: the Bogalusa Heart Study. *Metabolism* 1996;45:235-40.
8. Dabelea D, Bell RA, D'Agostino RB, Jr., et al. Incidence of diabetes in youth in the United States. *JAMA* 2007;297:2716-24.
9. Liese AD, D'Agostino RB, Jr., Hamman RF, et al. The burden of diabetes mellitus among US youth: prevalence estimates from the SEARCH for Diabetes in Youth Study. *Pediatrics* 2006;118:1510-8.
10. Fagot-Campagna A, Pettitt DJ, Engelgau MM, et al. Type 2 diabetes among North American children and adolescents: an epidemiologic review and a public health perspective. *J Pediatr* 2000;136:664-72.
11. Weiss R, Dziura J, Burgert TS, et al. Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med* 2004;350:2362-74.
12. Burgert TS, Taksali SE, Dziura J, et al. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. *J Clin Endocrinol Metab* 2006;91:4287-94.
13. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006;118:1388-93.
14. Dwyer T, Magnussen CG, Schmidt MD, et al. Decline in physical fitness from childhood to adulthood associated with increased obesity and insulin resistance in adults. *Diabetes Care* 2009;32:683-7.
15. Nader PR, Bradley RH, Houts RM, McRitchie SL, O'Brien M. Moderate-to-vigorous physical activity from ages 9 to 15 years. *JAMA* 2008;300:295-305.
16. Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, McDowell M. Physical activity in the United States measured by accelerometer. *Med Sci Sports Exerc* 2008;40:181-8.
17. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006;444:881-7.
18. Sunehag AL, Toffolo G, Campioni M, Bier DM, Haymond MW. Effects of dietary macronutrient intake on insulin sensitivity and secretion and glucose and lipid metabolism in healthy, obese adolescents. *J Clin Endocrinol Metab* 2005;90:4496-502.
19. Ellis KJ, Abrams SA, Wong WW. Monitoring childhood obesity: assessment of the weight/height index. *Am J Epidemiol* 1999;150:939-46.
20. Freedman DS, Ogden CL, Berenson GS, Horlick M. Body mass index and body fatness in childhood. *Curr Opin Clin Nutr Metab Care* 2005;8:618-23.

21. Shypailo RJ, Butte NF, Ellis KJ. DXA: can it be used as a criterion reference for body fat measurements in children? *Obesity (Silver Spring)* 2008;16:457-62.
22. Manson JE, Nathan DM, Krolewski AS, Stampfer MJ, Willett WC, Hennekens CH. A prospective study of exercise and incidence of diabetes among US male physicians. *JAMA* 1992;268:63-7.
23. Manson JE, Hu FB, Rich-Edwards JW, et al. A prospective study of walking as compared with vigorous exercise in the prevention of coronary heart disease in women. *N Engl J Med* 1999;341:650-8.
24. Tomeo CA, Colditz GA, Willett WC, et al. Harvard Report on Cancer Prevention. Volume 3: prevention of colon cancer in the United States. *Cancer Causes Control* 1999;10:167-80.
25. Caranti DA, de Mello MT, Prado WL, et al. Short- and long-term beneficial effects of a multidisciplinary therapy for the control of metabolic syndrome in obese adolescents. *Metabolism* 2007;56:1293-300.
26. Savoye M, Shaw M, Dziura J, et al. Effects of a weight management program on body composition and metabolic parameters in overweight children: a randomized controlled trial. *JAMA* 2007;297:2697-704.
27. Gutin B, Barbeau P, Owens S, et al. Effects of exercise intensity on cardiovascular fitness, total body composition, and visceral adiposity of obese adolescents. *Am J Clin Nutr* 2002;75:818-26.
28. Shaibi GQ, Cruz ML, Ball GD, et al. Effects of resistance training on insulin sensitivity in overweight Latino adolescent males. *Med Sci Sports Exerc* 2006;38:1208-15.

Chapter 2

A 12 week aerobic exercise program reduces hepatic fat accumulation and insulin resistance in obese, Hispanic adolescents

Gert-Jan van der Heijden
Zhiyue J. Wang
Zili Chu
Pieter J.J. Sauer
Morey W. Haymond
Luisa M. Rodriguez
Agneta L. Sunehag

Obesity (Silver Spring) (E-pub ahead of print). 2009 PMID 19696755

Abstract

Background: The rise in obesity related morbidity in children and adolescents requires urgent prevention and treatment strategies. Currently, only limited data are available on the effects of exercise programs on insulin resistance and visceral, hepatic and intramyocellular fat accumulation.

Objective: To test the hypothesis that a 12 wk controlled aerobic exercise program without weight loss reduces visceral, hepatic and intramyocellular fat content and decreases insulin resistance in sedentary Hispanic adolescents.

Participants and Design: Twenty-nine post-pubertal (Tanner IV-V), Hispanic adolescents, 15 obese (7 m, 8 f; 15.6±0.4 y; 33.7 ± 1.1 kg/m^2; 38.3±1.5% body fat) and 14 lean (10 m, 4 f; 15.1 ± 0.3 y; 20.6 ± 0.8 kg/m^2; 18.9 ± 1.5% body fat), completed a 12 wk aerobic exercise program (4 x 30 min/wk at ≥ 70% of VO_{2peak}). Measurements of cardiovascular fitness, visceral, hepatic and intramyocellular fat content (MRI/MRS) and insulin resistance were obtained at baseline and post-exercise.

Results: In both groups, fitness increased (obese: 13 ± 2% lean: 16 ± 4%; both $p < 0.01$). In obese participants, intramyocellular fat remained unchanged while hepatic fat content decreased from 8.9 ± 3.2 to 5.6 ± 1.8%; $p < 0.05$ and visceral fat content from 54.7 ± 6.0 to 49.6 ± 5.5 cm^2; $p < 0.05$. Insulin resistance decreased indicated by decreased fasting insulin (21.8 ± 2.7 to 18.2 ± 2.4 μU/mL; $p < 0.01$) and HOMA-IR (4.9±0.7 to 4.1 ± 0.6; $p < 0.01$). The decrease in visceral fat correlated with the decrease in fasting insulin ($R^2 = 0.40$; $p < 0.05$). No significant changes were observed in any parameter in lean participants except a small increase in lean body mass.

Conclusion: A controlled aerobic exercise program, without weight loss, reduced hepatic and visceral fat accumulation and decreased insulin resistance in obese adolescents.

Introduction

The prevalence of obesity and obesity related illnesses has increased dramatically in children and adolescents over the past decades. About 17% of American youth are obese (1). Of those that are obese, 30-50% have the metabolic syndrome (2), 30-40% have hepatic steatosis (3,4) and 15-30% of all newly diagnosed diabetic adolescents have obesity related type 2 diabetes (5,6). This is a very serious public health concern requiring urgent prevention and treatment strategies.

Lifestyle interventions including various combinations of diet, exercise and education programs have been shown to reduce obesity and its co-morbidities (7-9). It is, however, difficult to determine which interventions are most efficient and best accepted by children and adolescents. Weight loss improves metabolic disturbances but long term compliance is often unsuccessful, resulting in weight regain. Improved insulin sensitivity and reduced abdominal fat accumulation have been reported in response to exercise with and without weight loss in children and adolescents (10-15). However, potential confounders such as age and pubertal stage of the participants, duration, intensity and type of exercise and control of the diet preceding the pre- and post exercise measurements were often not well described. In addition, the effect of exercise on hepatic and intramyocellular fat were not measured. The purpose of the present study was to determine the effects of a controlled, moderately intensive aerobic exercise program that would be acceptable to sedentary obese and lean adolescents. No additional dietary and lifestyle advice was provided and there was no intent of weight loss. The study focused on Hispanic adolescents because of their high prevalence of obesity and its co-morbidities (1,4,16). A group of sedentary lean adolescents was included since sedentary lifestyle in itself might have negative metabolic effects (17). Further, effects of an exercise program in lean sedentary adolescents have not previously been reported.

We hypothesized that a 12 wk controlled aerobic exercise program without weight loss reduces visceral, hepatic and intramyocellular fat (IMCL) content and decreases insulin resistance in sedentary Hispanic adolescents.

Methods and Procedures

Participants

After approval of the protocol by the Baylor College of Medicine Institutional Review Board for Human Subject Research, and the General Clinical Research Center Advisory Board, lean and obese adolescents were recruited by local advertisement. Adolescents were screened and enrolled in the study after written assent from the participant and consent from the legal guardian were obtained.

Twenty nine (29) post pubertal (i.e. Tanner pubertal stage IV-V), Hispanic adolescents, 15 obese and 14 lean, were studied **(Table 1)**. All obese participants had BMI > 95th and all lean participants < 85th percentile for age according to CDC growth charts (18). Participants had been lean or obese for ≥ 5 years and reported stable body weight for at least 6 months. Only sedentary adolescents were included, defined by no school organized athletic program participation and < 45 min light to moderate physical activity/week. All participants were Hispanic (parents and grandparents of Hispanic descent by self report). The participants were in good health as determined by a medical history, a physical examination and a standard blood chemistry analysis including blood lipids, liver- and kidney function tests, hemoglobin, hematocrit, hemoglobin A1c and fasting and 2 h post-prandial glucose response. Participants were taking no medications including birth control pills and had no first-degree relatives with diabetes. Adolescents with morbid obesity (body fat % > 50, sleep apnea, Pickwick syndrome or cor pulmonale) were excluded.

Study Design

Each participant was studied on two separate occasions: 1) The weekend before start of the exercise program (baseline), 2) Three days after the final exercise session of the 12 wk exercise program (post). All procedures were identical on both study occasions.

To exclude effects of dietary intake on measurements obtained at baseline versus post-exercise, prior to both studies, each participant received an identical 7 d low-carbohydrate (CHO)/high-fat diet at home (30% CHO, 55% fat, and 15% protein; 20% of the total CHO content as fructose) (19-21). Total energy intake was calculated to correspond to each individual's requirement according to the Institute of Medicine Dietary Reference Intakes (22). Non-consumed food was examined for constituents, and the energy and macronutrient composition of the consumed food was calculated by

difference (19-21). In order to measure the effect of exercise alone, participants were told not to make lifestyle and dietary changes during the exercise program. On d 7 of the diet period, the participants were admitted to the Metabolic Research Unit (MRU) at the Children's Nutrition Research Center, Houston. Participants were fasted overnight for 12 h (except for water). Following the fast, four blood samples were obtained for measurements of glucose, insulin and alanine aminotransferase (ALT) concentrations. Subsequently, the participants were transferred to the radiology department at Texas Children's Hospital for Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) of abdominal, hepatic and intramyocellular fat content.

Table 1. Participant characteristics (mean ± SE)

	Obese		Lean		Interaction (p-value)$^{\$}$
	Baseline	Post	Baseline	Post	
N	15		14		
Male/female	7/8		10/4		
Age	15.6 ± 0.4		15.1 ± 0.3		
Tanner Stage IV/V	3/12		5/9		
Weight (kg)	91.7 ± 3.5	91.2 ± 3.5	57.2 ± 2.7$^{\#\#\#}$	58.0 ± 2.9$^{*\#\#\#}$	0.083
BMI (kg/m^2)	33.7 ± 1.1	33.4 ± 1.1	20.6 ± 0.8$^{\#\#\#}$	20.7 ± 0.8$^{\#\#\#}$	0.175
Body fat %	38.3 ± 1.5	37.3 ± 1.6*	18.9 ± 1.5$^{\#\#\#}$	18.6 ± 1.6$^{\#\#\#}$	0.286
Lean body mass (kg)	54.6 ± 2.7	55.3 ± 2.8	44.7 ± 2.3$^{\#\#}$	46.0 ± 2.4$^{*\#}$	0.409
Fat mass (kg)	35.2 ± 2.0	34.2 ± 2.1*	10.9 ± 1.0$^{\#\#\#}$	10.9 ± 1.1$^{\#\#\#}$	0.048
Glucose (mmol/L)	5.0 ± 0.1	5.0 ± 0.1	5.1 ± 0.1	5.0 ± 0.1	0.696
Insulin (μU/mL)	21.8 ± 2.7	18.2 ± 2.4***	7.3 ± 0.9$^{\#\#\#}$	6.7 ± 0.9$^{\#\#\#}$	0.001
HOMA-IR	4.9 ± 0.7	4.1 ± 0.6***	1.7 ± 0.2$^{\#\#\#}$	1.5 ± 0.2$^{\#\#\#}$	0.003
ALT (U/L)	39 ± 4	35 ± 3	27 ± 1$^{\#\#}$	27 ± 2	0.083
VO_2 peak L/min	2.45 ± 0.15	2.75 ± 0.18***	2.17 ± 0.14	2.47 ± 0.12**	0.975

Different from baseline within each group: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Different between obese and lean participants: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$.

$^{\$}$ANOVA 2-way interaction

Exercise Program

For the duration of 12 wks, participants came to the physical therapy unit at Texas Children's Hospital twice a week for a 30 min aerobic exercise session on a treadmill, elliptical or a bicycle (dependent on the preference of the participant). Each exercise session was preceded by 10 min of warm up and stretching and followed by 10 min

of cool down and stretching. The exercise intensity level was designed to result in a heart rate corresponding to at least 70% of that obtained at $VO_{2\ peak}$ at baseline (see below), i.e., we aimed at maintaining heart rates >140 beats/min. Experienced exercise physiologists were responsible for the training sessions together with the principal investigator. Participants were instructed to perform a similar program (same duration and intensity) twice a week at home, i.e., a total of 4 exercise sessions per week, which amounted to 48 sessions in the total exercise program. To assure that the desired heart rate (exercise intensity) was achieved and maintained for 30 min, each participant wore a heart rate monitor, Polar S-710 (Health Check Systems, Brooklyn, NY) during all home and hospital exercise sessions. Information from the monitors was downloaded and evaluated on a weekly basis. Participants performed no exercise outside the program. The participants' weight was assessed twice a week in conjunction with the exercise sessions to assure weight stability.

To avoid the acute effect of exercise on measurements obtained during the post exercise study, the last exercise session took place three days prior to the glucose, insulin and MRI/MRS measurements.

Cardiovascular Fitness

Peak oxygen consumption ($VO_{2\ peak}$) was measured at baseline and post-exercise using a modified Bruce treadmill protocol. The treadmill test started at a speed of 1.7 mph. Subsequently, the speed and incline was gradually increased every 3 min until maximal exercise capacity of the participant was reached. Oxygen consumption was measured with a Vmax-229 metabolic cart (Sensormedics, Anaheim, CA). $VO_{2\ peak}$ was determined using standard criteria, specifically a heart rate >195 beats/min or a respiratory quotient (RQ) > 1.0 at peak exercise (21).

Body Composition

Non-bone lean body (LBM) and fat mass (FM) were measured by dual-energy x-ray absorptiometry (DXA) (QDR 11.2; Hologic Bedford, MA) (19-21).

Abdominal fat content was measured by magnetic resonance imaging (MRI) and intrahepatic and intramyocellular lipid (IMCL) content (soleus muscle) by magnetic resonance spectroscopy (MRS) using a Philips Achieva 1.5T whole body clinical scanner, Software Release 1.5, Best, Holland.

The MR image of abdominal fat i.e. visceral (intra abdominal) and subcutaneous (peripheral) fat content, was acquired in a single transversal slice at the level of the

umbilicus as previously described (19,21). MRI data are expressed as cross-sectional area (cm^2).

A PRESS single voxel technique was used to obtain the liver MR spectra in a 3 x 3 x 3 cm^3 voxel, with repetition time (TR)/echo time (TE) = 5000/31 ms, NSA = 32 and water suppression off. Data were analyzed using the scanner software to obtain the peak areas of water and lipids, respectively. The lipid signals were treated as one composite peak, and integrated from 0.0 to 2.5 ppm after baseline correction. The result was expressed as the total lipid/water peak area ratio (%) (23). Hepatic fat was considered normal if the MRS lipid peak/water peak was <5.6 % and high if the MRS lipid peak/ water peak was > 5.6 % (24) **(Figure 1)**.

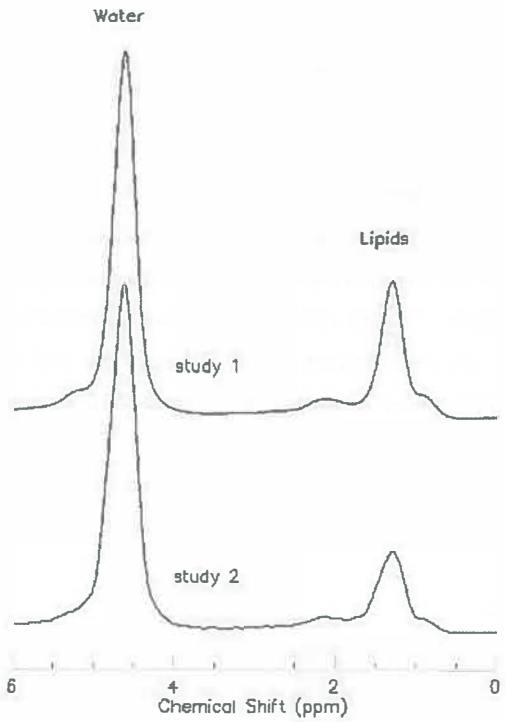

Figure 1. Representative liver spectra of an obese participant at baseline (study 1) and post-exercise (study 2).

A PRESS chemical shift imaging technique was used for measuring IMCL, with TR/ TE = 1500/31 ms, field of view = 120-160 mm, slice thickness = 10 mm, in-plane nominal voxel size 5 x 5 mm^2, number of signal average = 1, and no water suppression. Pre-saturation MREST pulses were used to suppress the lipid signal from outside the region of interest (ROI). The ROI excluded tibia and fibula bone marrow and was positioned at the same height and location for both study occasions. The spectral map was overlaid on a T1 weighted localizer image using the "SpecTool" software (version 4.5, provided by Philips Medical Systems). Our MR spectroscopic imaging method provided high spatial resolution data with good shim. There were several voxels inside the soleus muscle but only spectra from voxels showing well defined separation between EMCL and IMCL peaks were analyzed individually using jMRUI v3.0 (25) with the AMARES algorithm to obtain the peak areas as described by Szczepaniak et al. (26). The water and IMCL signals were quantified together from spectra acquired without water suppression. The average value of the IMCL/water ratio (%) from these spectra was used for statistical analysis.

Blood Sampling and Analyses

Blood samples were collected in EDTA tubes. Immediately after blood collection, samples were put on ice and span at 3000 rpm for 10 minutes. Plasma was stored at -80°C until final analysis. Glucose concentrations were measured using an enzymatic specific method (YSI glucose analyzer, Yellow Springs, OH); and insulin concentrations by electrochemiluminescence, using a Roche ElecsysÒ 1010 analyzer (Roche Diagnostics Corporation, Indianapolis, IN).

Insulin resistance was calculated by the homeostasis model assessment, HOMA-IR (fasting insulin µU/ml x fasting glucose mmol/l /22.5) (27).

Statistical Methods

Data are presented as mean ± SE. Repeated measures ANOVA (SPSS 17.0) was used to assess the effects of exercise, groups (lean vs. obese participants and obese participants with high hepatic fat vs. normal hepatic fat), and the interaction between groups and exercise. Unpaired t-test was used to assess differences between groups at baseline and post the exercise program after detection of interaction. Within group differences (i.e. baseline vs. post exercise) were assessed by paired t-test. Regression analysis was used to test for correlations between variables. A $p < 0.05$ was considered statistically significant.

Results

Obese and Lean Participants

Cardiovascular Fitness

Obese and lean participants completed 91 ± 2% and 87 ± 2% of the total exercise program (48 sessions), respectively (ns), at 86 ± 2% and 85 ± 1% of their heart rate at baseline $VO_{2\ peak}$, respectively (ns). Total $VO_{2\ peak}$ increased by 13 ± 2% in obese ($p = 0.0002$) and 16 ± 4% in lean adolescents ($p = 0.002$) **(Table 1)**.

Energy Intake

Prior to both study occasions, dietary compliance was not different in both lean and obese participants. Total energy intake was virtually identical on both study occasions in the obese (baseline: 2725 ± 165; Post: 2695 ± 170 kcal/d) and in the lean participants (baseline: 2318 ± 49; Post: 2314 ± 81 kcal/d). The macronutrient distribution was also identical on both study occasions and corresponded completely to that designed.

Baseline Comparison of Obese versus Lean Participants

In addition to higher body weight, BMI, body fat %, body fat mass and lean body mass **(Table 1)**, obese participants had higher subcutaneous, visceral, hepatic and intramyocellular fat content as compared to lean participants **(Figure 2)**.

While both obese and lean participants were normoglycemic, with no difference in plasma glucose concentration between the groups, obese participants had three times higher fasting insulin concentration and HOMA-IR, indicating they were more insulin resistant **(Table 1)**.

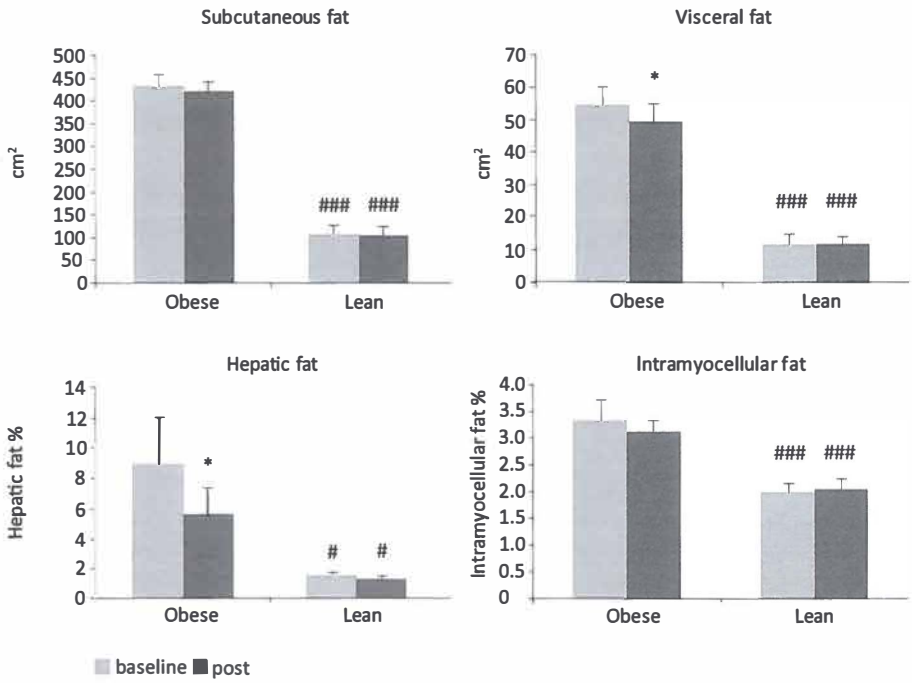

Figure 2. Subcutaneous, visceral, hepatic and intramyocellular fat content at baseline and post the exercise program (mean ± SE).

Different from baseline within each group: $^{*}p < 0.05$.

Different between obese and lean participants: $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$

Effects of the Exercise Program in *Obese* Participants

In response to the exercise program, total body weight, BMI and lean body mass did not change significantly. There was, however, a small but significant decrease in fat mass ($p = 0.03$) and body fat % ($p = 0.02$) **(Table 1)**.

Abdominal fat distribution

Visceral fat content decreased ($p = 0.03$) while subcutaneous fat content remained unchanged (**Figure 2**).

Hepatic fat content

In the whole group of obese participants, hepatic fat content decreased ($p = 0.04$) **(Figure 2)**. This change was primarily attributed to the decrease in hepatic fat content

in the 5 participants (33%) with high hepatic fat content, in whom hepatic fat decreased by 40, 44, 64, 42 and 26%, respectively. At baseline, participants with high hepatic fat had higher visceral fat content (69.4 ± 6.2 cm^2) compared to participants with normal hepatic fat content (47.3 ± 7.5 cm^2) (p = 0.04).

In the whole group of obese participants, hepatic fat content was directly correlated with ALT concentrations (Baseline: R^2 = 0.56; p = 0.03; Post: R^2 = 0.67; p = 0.008). Only in the participants with high hepatic fat, did the exercise related decrease in ALT concentration reach significance (Baseline: 47 ± 8; Post: 41 ± 8 U/L; p = 0.04).

Intramyocellular fat content

The exercise program did not have any effect on IMCL **(Figure 2).**

Insulin resistance

Fasting plasma glucose concentration did not change in response to the exercise program. Fasting plasma insulin concentration and HOMA-IR decreased in all obese participants (p = 0.0001 and p = 0.0007, respectively), indicating decreased insulin resistance **(Table 1)**. The decrease in HOMA-IR was a result of the decrease in insulin concentration. Therefore, we chose to use fasting insulin concentration to represent insulin resistance in all correlation analyses.

Fasting insulin concentration was directly correlated with visceral fat content (Baseline: R^2 = 0.47; p = 0.005; Post: R^2 = 0.52; p = 0.003), and the reduction in insulin concentration correlated with the decrease in visceral fat content (R^2 = 0.40; p = 0.01).

In addition, fasting insulin concentration correlated with hepatic fat content (both at baseline and post-exercise: R^2 = 0.31; p = 0.03). Participants with high hepatic fat content had higher insulin concentrations (Baseline: 33.03 ± 3.40; Post: 27.15 ± 3.84 μU/mL) compared to participants with normal hepatic fat content (Baseline: 16.20 ± 2.03; Post: 13.75 ± 1.80 μU/mL) (Baseline: p = 0.0006; Post p = 0.003). Insulin concentration did not correlate with IMCL.

Effects of the Exercise Program in *Lean* Participants

Except for a small but significant weight increase (p = 0.04), accounted for by an increase in lean body mass (p = 0.01), fat mass, body fat %, and visceral, subcutaneous, hepatic and intramyocellular fat content did not change significantly in response to the exercise program **(Table 1** and **Figure 2)**. In addition, plasma glucose, insulin and ALT concentrations did not change significantly. HOMA-IR decreased in 10/14 of the lean

participants, while it increased in 4. This points to an improvement in insulin sensitivity although the overall decrease in HOMA-IR did not reach significance ($p = 0.09$)

Discussion

The present study demonstrates that a twelve week aerobic exercise program (four times thirty minutes per week) without weight loss or change in BMI, results in increased cardiovascular fitness, reduced visceral and hepatic fat content and decreased insulin resistance in sedentary, post pubertal, obese, Hispanic adolescents. Subcutaneous and intramyocellular fat content remained unchanged.

Both obese and lean adolescents complied very well with the designed program with no difference between the groups. As a result, both groups increased their fitness to the same extent in response to the program.

In sedentary *lean* participants, the exercise program did not significantly change body composition (total fat mass, percent body fat and subcutaneous, visceral, hepatic and intramyocellular fat content) except for a small but significant increase in body weight due to an increase in lean body mass. Although insulin resistance measured by HOMA-IR decreased in 10/14 lean participants, the change in the whole lean group did not reach significance. In a recent review, Shaibi et al. (28) discussed that increased cardiovascular fitness might have an independent effect on decreasing insulin resistance, especially in boys (28). Thus, the increased fitness is an important result of the exercise program in these sedentary lean adolescents.

In *obese* participants, in addition to increased fitness, the exercise program (without weight loss) resulted in a substantial decrease in hepatic fat, particularly in adolescents with high hepatic fat content. The high prevalence of fatty liver in obese adolescents (3,4) and its potential to progress to liver inflammation, fibrosis and cirrhosis (non alcoholic fatty liver disease) (29) is a great concern. Our results indicate that aerobic exercise might be an effective way to counteract the development of this harmful disease. To our knowledge, these are the first data on the effect of an exercise program (without additional interventions) on hepatic fat content in children and adolescents. Only a couple of studies in adults have previously addressed this issue (30-32). Perseghin et al. (30) showed reduced prevalence of fatty liver in more as compared to less physically active individuals (30). In addition, Larson-Meyer et al. (31) found that 6 months caloric restriction with or without exercise decreased liver fat content. The findings of both

studies (30,31) are in agreement with our results. In contrast, Devries et al. (32) reported no effects on hepatic fat content and insulin sensitivity in response to a 12 wk aerobic exercise program with gradual increase from light to moderate intensity. The intensity of the exercise program in the Devries study (32) might have been insufficient to affect liver fat content.

Intramyocellular fat content remained unchanged in response to the exercise program despite decreased insulin resistance. In adults, reports on the relationship between intramyocellular fat content and insulin resistance are inconclusive (33). In children and adolescents, a direct correlation has been reported between insulin resistance and intramyocellular fat (34-36). We did not observe any correlation between insulin resistance and intramyocellular fat content. However, intramyocellular fat content was significantly higher (Baseline 68%; Post 53%) in the insulin resistant obese as compared with the insulin sensitive lean participants. Kelley and Mandarino described that a failure to increase lipid oxidation during fasting leads to intramyocellular fat deposition in obese individuals, subsequently contributing to patterns of insulin resistance (37). In agreement with our results, Bruce et al. (38) reported decreased insulin resistance despite unchanged intramyocellular fat content after exercise training in obese adults. A possible explanation for this paradox is that exercise increases the oxidative capacity in the muscles and intramyocellular lipid is used as a fuel source (38,39).

Several investigators have measured the effect of aerobic exercise programs on insulin resistance and body composition in children. Nassis et al. (10) reported that a 12 wk aerobic exercise program (3 x 40 min/wk; Average exercise intensity: HR 161 ± 2 beats/min) without weight loss, decreased insulin resistance in sedentary overweight and obese girls (9-15 y). Similarly, Bell et al. (11) reported that an 8 wk combined aerobic and resistance exercise program (3 x 60 min/wk) without weight loss, resulted in decreased insulin resistance and reduced waist circumference in sedentary obese children and adolescents (9-16 y). Although both studies (10,11) differed from ours with regard to the pubertal stage of the participants, the effects of exercise on insulin resistance were similar to our results. However, diets preceding the measurements and physical activity outside the program were not controlled, and abdominal, hepatic and intramyocellular fat content were not measured. Finally, the exercise program by Bell et al. (11) included both aerobic and resistance exercise, thus, precluding evaluation of the individual effects of these two different types of exercise.

With regard to body composition, Gutin et al. (12) observed that in 7-11 y old obese children, a 12 wk aerobic exercise program (~4 x 40 min/wk at HR ~157 beats/min) attenuated growth related increase in visceral fat accumulation in comparison to a non exercising control group. In another study in obese adolescents (13-16 y), Gutin et al. (13) demonstrated that an 8 month program of physical activity (> 2 x/wk at moderate or vigorous intensity) combined with lifestyle education (1 h sessions every 2 wks) decreased visceral fat content. Both study results are in line with our findings. Our results indicate that exercise without weight loss results in decreased visceral but not subcutaneous fat content. We postulate that this is due to the fact that visceral fat is more metabolically active (40).

In the obese participants, fasting insulin concentration was strongly and consistently correlated with visceral fat content but not with body weight, BMI, body fat % or total, subcutaneous or intramyocellular fat content. In addition, the decrease in visceral fat content resulting from the exercise program was significantly correlated with the decrease in insulin concentration. The results from other published studies investigating the relationship between abdominal fat distribution and insulin sensitivity in children and adolescents are conflicting (36,41-44). Some studies have found a relationship between insulin sensitivity and visceral fat (36,43), while other have reported a relation between insulin sensitivity and subcutaneous fat (41,42) or with both visceral and subcutaneous fat deposits (44). One possible reason for these inconsistencies might be differences in pubertal stage of the participants. However, the mechanism for the contribution from visceral and subcutaneous fat to insulin resistance is still an unresolved issue. It has been speculated that defective differentiation of subcutaneous fat and/or increased inflammation in visceral fat might be involved (40,45).

We also demonstrated that in the whole group of obese participants, fasting insulin concentration was directly correlated with hepatic fat content. Further, the obese participants with high hepatic fat were significantly more insulin resistant than those with normal hepatic fat content. This is in agreement with reports by Burgert et al. (3) and Deivanayagam et al. (46). In addition, the latter group (46) observed that obese adolescents with hepatic steatosis had higher visceral fat content compared with those without hepatic steatosis. Overall, our data indicate a metabolic interaction between abdominal fat, specifically visceral and hepatic fat, and insulin resistance.

In summary, our results demonstrate that sedentary, post pubertal, obese and lean, Hispanic adolescents comply very well with a controlled 12 wk aerobic exercise program (4 x 30 min/wk at a heart rate corresponding to ~85% of $VO_{2\ peak}$) without additional

dietary and lifestyle advice or weight loss. In both lean and obese participants, the program resulted in increased fitness. In addition, in obese participants, the program resulted in decreased visceral and hepatic fat accumulation and decreased insulin resistance. More research is warranted to investigate the promising potential of exercise to prevent and treat non alcoholic fatty liver disease in obese adolescents.

References

1. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. *Jama* 2006;295:1549-55.
2. Weiss R, Dziura J, Burgert TS, et al. Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med* 2004;350:2362-74.
3. Burgert TS, Taksali SE, Dziura J, et al. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. *J Clin Endocrinol Metab* 2006;91:4287-94.
4. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006;118:1388-93.
5. Liese AD, D'Agostino RB, Jr., Hamman RF, et al. The burden of diabetes mellitus among US youth: prevalence estimates from the SEARCH for Diabetes in Youth Study. *Pediatrics* 2006;118:1510-8.
6. Fagot-Campagna A, Pettitt DJ, Engelgau MM, et al. Type 2 diabetes among North American children and adolescents: an epidemiologic review and a public health perspective. *J Pediatr* 2000;136:664-72.
7. Dao HH, Frelut ML, Oberlin F, Peres G, Bourgeois P, Navarro J. Effects of a multidisciplinary weight loss intervention on body composition in obese adolescents. *Int J Obes Relat Metab Disord* 2004;28:290-9.
8. Savoye M, Shaw M, Dziura J, et al. Effects of a weight management program on body composition and metabolic parameters in overweight children: a randomized controlled trial. *Jama* 2007;297:2697-704.
9. Caranti DA, de Mello MT, Prado WL, et al. Short- and long-term beneficial effects of a multidisciplinary therapy for the control of metabolic syndrome in obese adolescents. *Metabolism* 2007;56:1293-300.
10. Nassis GP, Papantakou K, Skenderi K, et al. Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. *Metabolism* 2005;54:1472-9.
11. Bell LM, Watts K, Siafarikas A, et al. Exercise alone reduces insulin resistance in obese children independently of changes in body composition. *J Clin Endocrinol Metab* 2007;92:4230-5.
12. Gutin B, Owens S. Role of exercise intervention in improving body fat distribution and risk profile in children. *Am J Hum Biol* 1999;11:237-47.
13. Gutin B, Barbeau P, Owens S, et al. Effects of exercise intensity on cardiovascular fitness, total body composition, and visceral adiposity of obese adolescents. *Am J Clin Nutr* 2002;75:818-26.
14. Treuth MS, Hunter GR, Figueroa-Colon R, Goran MI. Effects of strength training on intra-abdominal adipose tissue in obese prepubertal girls. *Med Sci Sports Exerc* 1998;30:1738-43.
15. Shaibi GQ, Cruz ML, Ball GD, et al. Effects of resistance training on insulin sensitivity in overweight Latino adolescent males. *Med Sci Sports Exerc* 2006;38:1208-15.
16. Narayan KM, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. *JAMA* 2003;290:1884-90.
17. Ischander M, Zaldivar F, Jr., Eliakim A, et al. Physical activity, growth, and inflammatory mediators in BMI-matched female adolescents. *Med Sci Sports Exerc* 2007;39:1131-8.
18. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM et.al. CDC Growth Charts: United States. Advance Data, Center for Disease Control and Prevention, National Center for Health Statistics No 314, 2000:1-28.
19. Sunehag AL, Toffolo G, Campioni M, Bier DM, Haymond MW. Effects of dietary macronutrient intake on insulin sensitivity and secretion and glucose and lipid metabolism in healthy, obese adolescents. *J Clin Endocrinol Metab* 2005;90:4496-502.

20. Sunehag AL, Toffolo G, Treuth MS, et al. Effects of dietary macronutrient content on glucose metabolism in children. *J Clin Endocrinol Metab* 2002;87:5168-78.
21. Treuth MS, Sunehag AL, Trautwein LM, Bier DM, Haymond MW, Butte NF. Metabolic adaptation to high-fat and high-carbohydrate diets in children and adolescents. *Am J Clin Nutr* 2003;77:479-89.
22. Otten J, Pitzi Hellwig J, Meyers L. *Dietary (DRI) Reference Intakes: The Essential Guide to Nutrient Requirements*. The National Academies Press: Washington, DC, 2006.
23. May GL, Wright LC, Holmes KT, et al. Assignment of methylene proton resonances in NMR spectra of embryonic and transformed cells to plasma membrane triglyceride. *J Biol Chem* 1986;261:3048-53.
24. Szczepaniak LS, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005;288:E462-8.
25. Naressi A, Couturier C, Devos JM, et al. Java-based graphical user interface for the MRUI quantitation package. *MAGMA* 2001;12:141-52.
26. Szczepaniak LS, Babcock EE, Schick F, et al. Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. *Am J Physiol* 1999;276:E977-89.
27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-9.
28. Shaibi GQ, Roberts CK, Goran MI. Exercise and insulin resistance in youth. *Exerc Sport Sci Rev* 2008;36:5-11.
29. Molleston JP, White F, Teckman J, Fitzgerald JF. Obese children with steatohepatitis can develop cirrhosis in childhood. *Am J Gastroenterol* 2002;97:2460-2.
30. Perseghin G, Lattuada G, De Cobelli F, et al. Habitual physical activity is associated with intrahepatic fat content in humans. *Diabetes Care* 2007;30:683-8.
31. Larson-Meyer DE, Newcomer BR, Heilbronn LK, et al. Effect of 6-month calorie restriction and exercise on serum and liver lipids and markers of liver function. *Obesity (Silver Spring)* 2008;16:1355-62.
32. Devries MC, Samjoo IA, Hamadeh MJ, Tarnopolsky MA. Effect of Endurance Exercise on Hepatic Lipid Content, Enzymes, and Adiposity in Men and Women. *Obesity (Silver Spring)* 2008.
33. Corcoran MP, Lamon-Fava S, Fielding RA. Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise. *Am J Clin Nutr* 2007;85:662-77.
34. Sinha R, Dufour S, Petersen KF, et al. Assessment of skeletal muscle triglyceride content by (1)H nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. *Diabetes* 2002;51:1022-7.
35. Weiss R, Dufour S, Groszmann A, et al. Low adiponectin levels in adolescent obesity: a marker of increased intramyocellular lipid accumulation. *J Clin Endocrinol Metab* 2003;88:2014-8.
36. Liska D, Dufour S, Zern TL, et al. Interethnic differences in muscle, liver and abdominal fat partitioning in obese adolescents. *PLoS ONE* 2007;2:e569.
37. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. *Diabetes* 2000;49:677-83.
38. Bruce CR, Thrush AB, Mertz VA, et al. Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. *Am J Physiol Endocrinol Metab* 2006;291:E99-E107.
39. Pruchnic R, Katsiaras A, He J, Kelley DE, Winters C, Goodpaster BH. Exercise training increases intramyocellular lipid and oxidative capacity in older adults. *Am J Physiol Endocrinol Metab* 2004;287:E857-62.

40. Mathieu P, Poirier P, Pibarot P, Lemieux I, Despres JP. Visceral obesity: the link among inflammation, hypertension, and cardiovascular disease. *Hypertension* 2009;53:577-84.Gower BA, Nagy TR, Goran MI. Visceral fat, insulin sensitivity, and lipids in prepubertal children. *Diabetes* 1999;48:1515-21.
41. Maffeis C, Manfredi R, Trombetta M, et al. Insulin sensitivity is correlated with subcutaneous but not visceral body fat in overweight and obese prepubertal children. *J Clin Endocrinol Metab* 2008;93:2122-8.
42. Caprio S, Hyman LD, Limb C, et al. Central adiposity and its metabolic correlates in obese adolescent girls. *Am J Physiol* 1995;269:E118-26.
43. Taksali SE, Caprio S, Dziura J, et al. High visceral and low abdominal subcutaneous fat stores in the obese adolescent: a determinant of an adverse metabolic phenotype. *Diabetes* 2008;57:367-71.
44. McLaughlin T, Sherman A, Tsao P, et al. Enhanced proportion of small adipose cells in insulin-resistant vs insulin-sensitive obese individuals implicates impaired adipogenesis. *Diabetologia* 2007;50:1707-15.
45. Deivanayagam S, Mohammed BS, Vitola BE, et al. Nonalcoholic fatty liver disease is associated with hepatic and skeletal muscle insulin resistance in overweight adolescents. *Am J Clin Nutr* 2008;88:257-62.

Chapter 3

Aerobic exercise increases peripheral and hepatic insulin sensitivity in sedentary adolescents

Gert-Jan van der Heijden
Gianna Toffolo
Erica Manesso
Pieter J.J. Sauer
Agneta L. Sunehag

J Clin Endocrinol Metab (E-pub ahead of print) 2009 PMID 19808855

Abstract

Background: Data are limited on the effects of controlled aerobic exercise programs (without weight loss) on insulin sensitivity and glucose metabolism in children and adolescents.

Objective: To determine whether a controlled aerobic exercise program (without weight loss) improves peripheral and hepatic insulin sensitivity and affects glucose production (GPR), gluconeogenesis and glycogenolysis in sedentary lean and obese Hispanic adolescents.

Participants and Design: Twenty-nine post-pubertal adolescents (14 lean: 15.1 ±0.3y; 20.6 ± 0.8kg/m^2; 18.9 ± 1.5% body fat and 15 obese: 15.6 ± 0.4y; 33.2 ± 0.9kg/m^2; 38.4 ± 1.4% body fat) (mean±SE), completed a 12 wk aerobic exercise program (4x30 min/week at ^{3}70% of VO_2 peak). Peripheral and hepatic insulin sensitivity and glucose kinetics were quantified using GCMS pre- and post-exercise.

Results: No weight loss occurred. Lean and obese participants complied well with the program (~90% of the exercise sessions attended, resulting in ~15% increase in fitness in both groups). Peripheral and hepatic insulin sensitivity were higher in lean than obese adolescents but increased in both groups; peripheral insulin sensitivity by 35 ± 14% (lean) ($p < 0.05$) and 59 ± 19% (obese) ($p < 0.01$) and hepatic insulin sensitivity by 19 ± 7% (lean) ($p < 0.05$) and 23 ± 4% (obese) ($p < 0.01$). GPR, gluconeogenesis and glycogenolysis did not differ between the groups. GPR decreased slightly, 3 ± 1% (lean) ($p < 0.05$) and 4 ± 1% (obese) ($p < 0.01$). Gluconeogenesis remained unchanged, while glycogenolysis decreased slightly in the obese group ($p < 0.01$).

Conclusion: This well accepted aerobic exercise program, without weight loss, is a promising strategy to improve peripheral and hepatic insulin sensitivity in lean and obese sedentary adolescents. The small decrease in GPR is probably of limited clinical relevance.

Introduction

Reduced physical activity and increased sedentary behavior are risk factors for the development of obesity and many chronic diseases (1). Thus, the dramatic decline in physical activity between childhood and adolescence is a serious concern. Troiano et al. (1) reported that only 8% of adolescents (12-19y) obtained the recommended 60 min per day of exercise.

A number of studies in adults have demonstrated that regular moderate exercise improves insulin sensitivity and reduces the risk for cardiovascular disease, type 2 diabetes and some types of cancer (2-4). In addition, studies in adults have addressed effects of exercise on glucose kinetics (5,6) but the results are inconclusive. While Bergman et al. (5) demonstrated increased at rest gluconeogenesis from lactate in response to exercise, Coggan et al. (6) reported unchanged at rest gluconeogenesis (from pyruvate) and glycogenolysis while gluconeogenesis (from pyruvate) and glycogenolysis during exercise decreased. With regard to lipid metabolism, Phillips et al. (7) and Romijn et al. (8) demonstrated that intense and long term exercise increased lipid kinetics.

Improved insulin sensitivity has been shown in children and adolescents in response to various exercise programs (9-14). However, many exercise studies do not provide information about potential confounders such as attendance, intensity, physical activity outside the program and fitness at start of the program.

Further, to our knowledge no published studies have measured the effect of exercise on peripheral and hepatic insulin sensitivity separately, or reported the impact of training on glucose and lipid metabolism in children or adolescents.

The purpose of this study was to determine whether a controlled moderate aerobic exercise program (without weight loss or additional lifestyle education) improves peripheral and hepatic insulin sensitivity, and affects glucose production, gluconeogenesis, glycogenolysis and lipolysis in sedentary lean and obese Hispanic adolescents.

We focused on Hispanics because of their high risk of obesity and obesity related illnesses (15-17) and we included lean participants because a sedentary lifestyle per se is an additional risk factor (18).

Participants and Methods

Participants

After approval of the protocol by the Baylor College of Medicine Institutional Review Board for Human Subject Research, and the General Clinical Research Center Advisory Board, obese and lean adolescents were recruited by local advertisement. Adolescents were screened and enrolled in the study after written assent from the participant and consent from the legal guardian were obtained.

Twenty nine (29) post pubertal (Tanner IV-V) Hispanic adolescents (14 lean; 15 obese), were studied **(Table 1)**. The lean participants had BMI < 85th and the obese > 95th percentile for age (19). BMI might, however, not be an optimal marker of leanness/ obesity (20). Thus, to assure that our lean participants were indeed lean not only with regard BMI criteria but also to fatness, they must have < 27% body fat as measured by dual-energy x-ray absorptiometry (DXA) **(Table 1)**. Participants had been lean or obese for ≥ 5 years and reported stable body weight for at least 6 months. Only sedentary adolescents were included, i.e. they did not participate in any school or after school organized athletic activities and performed < 45 min light to moderate physical activity/ week.

Table 1. Demographic characteristics (mean ± SE)

	Lean participants		Obese participants		Interaction (p-value)[$]
	Baseline	Post	Baseline	Post	
male/ female	10/4	10/4	7/8	7/8	
Weight (kg)	57.2 ± 2.7	58.0 ± 2.9*	89.6 ± 3.2$^{\#\#}$	89.3 ± 3.1$^{\#\#}$	0.118
BMI (kg/m^2)	20.6 ± 0.8	20.7 ± 0.8	33.2 ± 0.9$^{\#\#}$	33.0 ± 0.8$^{\#\#}$	0.212
Body fat (%)	18.9 ± 1.5	18.6 ± 1.6	38.4 ± 1.5$^{\#\#}$	37.3 ± 1.5$^{\#\#**}$	0.187
Lean body mass (kg)	44.7 ± 2.3	46.0 ± 2.4**	53.3 ± 2.8$^{\#}$	54.4 ± 3.0$^{\#**}$	0.764
Fat mass (kg)	10.9 ± 1.0	10.9 ± 1.1	34.3 ± 1.5$^{\#\#}$	33.4 ± 1.4$^{\#\#*}$	0.062

Different from baseline within each group: * $p < 0.05$, ** $p < 0.01$.

Different between lean and obese participants: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

[$]GEE interaction between the effect of the exercise program and group

All participants were Hispanic (parents and grandparents of Hispanic descent by self report). The participants were in good health as determined by a medical history, a physical examination and a standard blood chemistry analysis including blood lipids,

liver- and kidney function tests, hemoglobin, hematocrit, hemoglobin A1c and fasting and 2 h post-prandial glucose response. Participants were taking no medications including birth control pills and had no first-degree relatives with diabetes. Adolescents with morbid obesity (body fat % > 50, sleep apnea, Pickwick syndrome or cor pulmonale) were excluded.

Study Design

Each participant was studied on two separate occasions: 1) The weekend before start of the exercise program (baseline), 2) Three days after the final exercise session of the 12 wk program (post). All procedures were identical on both study occasions.

To exclude effects of dietary intake on measurements obtained at baseline versus post-exercise, prior to both studies, each participant received an identical 7 d low-carbohydrate (CHO)/high-fat diet at home (30% CHO, 55% fat, and 15% protein; 20% of the total CHO content as fructose) (21-23). Total energy intake corresponded to each individual's requirement according to the Institute of Medicine Dietary Reference Intakes (24). The food was delivered to the participants' homes by the metabolic research kitchen. Non-consumed food was returned and examined for constituents and the energy and macronutrient content of the consumed food was calculated by difference (21-23). In order to measure the effect of exercise alone, participants were told not to make lifestyle and dietary changes during the exercise program.

On both occasions, the participants were admitted to the General Clinical Research Center at Texas Children's Hospital in the evening before the metabolic study. After dinner and a snack, participants were fasted overnight (except for water) i.e. from 2000 h until completion of the isotope infusion study at 1300 h the next day.

Exercise Program

For the duration of 12 wks, participants came to the physical therapy unit at Texas Children's Hospital twice a week for a 30 min aerobic exercise session on a treadmill, elliptical or bicycle (dependent on the participant's preference). Each exercise session was preceded and followed by 10 min of warm up/cool down and stretching. The exercise intensity level was designed to result in a heart rate corresponding to at least 70% of that obtained at $VO_{2\ peak}$ at baseline (see below), i.e., we aimed at heart rates > 140 beats/min for the entire 30 min session. Experienced exercise physiologists were responsible for the training sessions together with the principal investigator. No more than two participants were supervised at the same time. Flexibility with appointment

times and assistance with transportation when needed facilitated good attendance. Participants were instructed to perform a similar program (same duration and intensity) twice a week at home, i.e., a total of 4 exercise sessions per week. To assure that the desired heart rate (exercise intensity) was achieved and maintained for 30 min, each participant wore a heart rate monitor, Polar S-710 (Health Check Systems, Brooklyn, NY) during all home and hospital exercise sessions. Information from the monitors was downloaded and discussed with the participant on a weekly basis. Participants performed no exercise outside the program. Their weight was assessed twice a week in conjunction with the exercise sessions to assure weight stability. To avoid the acute effect of exercise on measurements obtained during the post exercise study, the last exercise session took place three days prior to the metabolic study

Tracers

Deuterium oxide (99% ^{2}H); [$^{2}H_5$]glycerol (99% [^{2}H], 95% [$^{2}H_5$]); [1-^{13}C]glucose (99% [^{13}C]); and [6,6-$^{2}H_2$]glucose (99% [^{2}H], 98% [$^{2}H_2$]) were purchased from Cambridge Isotope Laboratories (Andover, MA). The isotopes were tested for sterility and pyrogenicity by the investigation pharmacy at Texas Children's Hospital (Houston, TX). The infusates were filtered through a Millex GP syringe filter (0.22 µm; Millipore Corporation, Bedford, MN) and stored at 4°C for no more than 24-48 h before administration.

Administration of Tracers

On each study occasion, the participants received the following, stable isotopically labeled tracers as previously described (21,22,25).

1. During the overnight fast at 2100, 2300, 0100 and 0300 h, deuterium oxide (a total of 3 g/kg) was administered orally to measure total gluconeogenesis (26).
2. Between 0600 and 1300 h, a simultaneous, primed (60 x the minute infusion rate), constant rate i.v. infusion of [1-^{13}C]glucose (0.33 ± 0 µmol/kg $_{\text{non-bone lean body mass (LBM)}}$ · min) and [$^{2}H_5$]glycerol (0.14 ± 0 µmol/kg $_{\text{LBM}}$ · min) was administered to measure glucose production and the plasma turnover of glycerol, an indicator of lipolysis (21,22,25).
3. The Stable Label Intravenous Glucose Tolerance Test (SLIVGTT) was started at 0900 h after the 0 min blood sample (see below). A bolus injection of glucose, 0.35 ± 0 g/kg $_{\text{LBM}}$ containing 10% [6,6-$^{2}H_2$]glucose, was administered over 90-120 sec to measure insulin sensitivity (21,22,25).

Blood Sampling

Blood samples were obtained just before start of the primed constant rate infusion of the [1-^{13}C]glucose and [$^{2}H_5$]glycerol (designated as t = –180) (13 mL) and subsequently at t = -30, -20, -10, and 0 minutes (8 mL/sample). The injection of the SLIVGTT bolus (after the 0 min sample) was followed by blood sampling (3.6 mL per sample) at +2, 3, 4, 5, 8, 10, 18, 20, 23, 28, 32, 40, 60, 120, 180, and 240 min (21,22,25).

Analyses

Glucose concentrations were measured using an YSI glucose analyzer (Yellow Springs, OH) and insulin concentrations by electrochemiluminescence using a Roche Elecsys® 1010 analyzer (Roche Diagnostics Corporation, Indianapolis, IN). Plasma lipids were determined by standard laboratory techniques; Leptin and Adiponectin concentrations by non radioactive human ELISA kits (Linco Research, Inc., St Charles, MO), and high sensitive C-reactive protein (hs-CRP) by immunoturbidimetry. Non-bone lean body (LBM) and fat mass (FM) were measured by DXA (QDR 11.2; Hologic Bedford, MA) (21-23). Cardiovascular fitness was determined at baseline and post-exercise by peak oxygen uptake ($VO_{2\ peak}$) using a modified Bruce treadmill protocol (starting at a speed of 1.7 mph with subsequent increase of speed and incline every 3 min until the participant's maximal exercise capacity was reached). Oxygen consumption was measured with a Vmax-229 metabolic cart (Sensormedics, Anaheim, CA). $VO_{2\ peak}$ was determined using standard criteria, specifically a heart rate > 195 beats/min or a respiratory quotient (RQ) > 1.0 at peak exercise (23).

Calculations

Rates of glucose production and glycerol turnover were calculated under approximate steady-state conditions from the average isotopic enrichments obtained for [$^{13}C_1$] glucose and [$^{2}H_5$]glycerol, respectively, in the samples obtained at -30, -20, -10 and 0 min (21,22,25).

During the same period, the gluconeogenic contribution to glucose (GNG) was determined using $^{2}H_2O$ and the average ^{2}H enrichments of carbons 1,3,4,5,and 6 of glucose as recently published (26).

Peripheral insulin sensitivity (the sensitivity of glucose disposition to insulin) was calculated by applying the minimal model to SLIVGTT data (21,22, 25,27).

Hepatic insulin sensitivity was calculated in the fasting state by the hepatic insulin sensitivity index (HISI): 1000/(GPR [μmol/kg $_{LBM}$· min) x fasting plasma insulin (μU/mL)],

where 1000 is a constant that results in numbers between 1 and 10, as described by Matsuda et al. (28).

Statistical Methods

Data are presented as mean ± SE. Generalized Estimating Equations (GEE) (SPSS 17.0) were used to assess the effects of the exercise program, group (lean vs. obese participants) and the interaction between the effects of the exercise program and group. Post hoc procedures provided by GEE were used to compare groups at baseline and post-exercise and to assess exercise effect within each group. A $p < 0.05$ was considered statistically significant. The data on peripheral insulin sensitivity were log transformed to make the distribution bell shaped.

Results

Demographic and Biochemical Characteristics at Baseline

Demographic characteristics of the participants are given in **Table 1**.

As expected, weight, BMI, body fat %, total fat mass and lean body mass were significantly higher in obese as compared to lean participants.

Biochemical characteristics of the participants are given in **Table 2**. While glucose concentrations were not different, insulin concentrations were higher in the obese participants ($p < 0.01$).

Dietary Intake

In both lean and obese adolescents, total energy intake at baseline and post-exercise were not different, Lean: Baseline 2318 ± 94; Post 2314 ± 88 kcal/d; Obese: Baseline 2706 ± 166; Post 2677 ± 171 kcal/d. Similarly, macronutrient distribution of the intakes was identical on both study occasions in both groups (30 ± 1% CHO, 54 ± 1% fat, 16 ± 1% protein).

Compliance

Compliance with and response to the exercise program were equal in lean and obese adolescents. They attended 87 ± 2 (lean) and 89 ± 2% (obese) of the total 48 sessions (ns), at an intensity of 85 ± 1 (lean) and 86 ± 1% (obese) of their heart rate at $VO_{2\ peak}$ (ns). $VO_{2\ peak}$ increased by ~16 % in the lean (Baseline: 2.17 ± 0.14; Post: 2.47 ± 0.12

l/min; $p < 0.01$) and by ~12% in the obese participants (Baseline: 2.46 ± 0.16; Post: 2.75 ± 0.18 l/min; $p < 0.01$).

Table 2. Biochemical characteristics (mean ± SE)

	Lean participants		Obese participants		Interaction (p-value)[$]
	Baseline	Post	Baseline	Post	
Glucose (mmol/L)	5.1 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	0.812
Insulin (µmol/L)	7.3 ± 0.9	6.7 ± 0.9	20.1 ± 2.5##	17.2 ± 2.1*##	0.005
Triglycerides (mg/dL)	68 ± 8	82 ± 9*	74 ± 10	73 ± 8	0.042
Free Fatty Acids (mmol/L)	0.46 ± 0.03	0.50 ± 0.04	0.47 ± 0.03	0.51 ± 0.04	0.924
LDL cholesterol (mg/dL)	85 ± 8	82 ± 7	82 ± 6	79 ± 5	0.975
HDL cholesterol (mg/dL)	52 ± 4	52 ± 4	40 ± 3##	42 ± 2#	0.531
Total cholesterol (mg/dL)	152 ± 8	152 ± 6	141 ± 6	140 ± 4	0.827
Adiponectin (µg/mL)	7.2 ± 0.9	6.7 ± 0.9	5.9 ± 0.7	5.7 ± 0.7	0.538
Leptin (ng/mL)	5.5 ± 1.4	5.9 ± 1.6	37.3 ± 4.0##	34.4 ± 4.7##	0.089
Hs-CRP (mg/L)	0.2 ± 0.0	0.4 ± 0.1	1.1 ± 0.3#	1.1 ± 0.4	0.806

Different from baseline within each group: * $p < 0.05$, ** $p < 0.01$.

Different between lean and obese participants: # $p < 0.05$, ## $p < 0.01$.

[$]GEE interaction between the effect of the exercise program and group

Effects of the Exercise Program

Body Composition

In lean participants, body weight increased (0.8 ± 0.3 kg, $p < 0.05$) due to an increase in lean body mass (1.3 ± 0.4 kg, $p < 0.01$) **(Table 1)**. In obese participants, body weight did not change. However, lean body mass increased (1.1 ± 0.4 kg, $p < 0.01$) and fat mass decreased to the same extent (1.0 ± 0.5 kg, $p < 0.05$) **(Table 1)**.

Insulin Sensitivity

Peripheral insulin sensitivity

Compared to lean, obese adolescents had lower peripheral insulin sensitivity (Baseline: $p < 0.01$; Post: $p < 0.05$) **(Figure 1)**. In both lean and obese participants, peripheral insulin sensitivity increased in response to the exercise program, 35 ± 14% ($p < 0.05$) and 59 ± 19% ($p < 0.01$), respectively. Percentage change in lean vs. obese participants NS.

Hepatic insulin sensitivity

Compared to lean, obese participants had lower hepatic insulin sensitivity (Baseline and Post: $p < 0.01$) **(Figure 1)**. In both lean and obese adolescents, hepatic insulin sensitivity increased in response to the exercise program, 19 ± 7% ($p < 0.01$) and 23 ± 4% ($p < 0.01$), respectively. Percentage change in lean vs. obese participants NS.

Glucose Production from Gluconeogenesis and Glycogenolysis

Glucose production rate (GPR) (μmol/kg $_{LBM}$·min), did not differ between lean and obese participants **(Figure 1)**. Similarly, the contribution of gluconeogenesis (GNG) and glycogenolysis (GLY) to GPR were also not different (Baseline: Lean: GNG: 56 ± 2%; GLY 44 ± 2%; Obese: GNG 58 ± 2%; GLY 42 ± 2%).

In both lean and obese adolescents, the exercise program resulted in a small decrease in GPR, 3 ± 1% ($p < 0.05$) and 4 ± 1% ($p < 0.01$), respectively. In the obese participants, this decrease was accounted for by an 8 ± 3% decrease in glycogenolysis ($p < 0.01$). In the lean participants, the small decreases in GNG (3 ± 2%) and GLY (2 ± 3%), did not reach significance.

Lipolysis

At baseline, total glycerol Rate of appearance (Ra) (μmol/min), was higher in obese as compared to lean adolescents ($p < 0.01$) **(Figure 1)**. Glycerol Ra, expressed per μmol/ kg $_{FM}$·min, was lower in obese participants (Baseline: Lean: 17.9 ± 3.0; Obese: 6.9 ± 0.6 μmol/kg $_{FM}$·min, $p < 0.01$).

Except for higher HDL cholesterol in the lean participants ($p<0.01$), baseline blood lipids did not differ between the two groups **(Table 2)**.

The exercise program did not significantly affect glycerol Ra in either lean or obese adolescents **(Figure 1)**. Except for a slight increase in triglyceride concentration (within normal range) ($p < 0.05$) in lean participants, blood lipids were also not affected by the program **(Table 2)**.

Plasma Adiponectin, Leptin and Hs-CRP

Adiponectin concentration did not differ between lean and obese adolescents, whereas leptin and hs-CRP were higher in obese participants (Leptin: baseline and post $p < 0.01$; hs-CRP: baseline $p < 0.01$, post ns) **(Table 2)**. The exercise program did not significantly change adiponectin, leptin or hs-CRP concentrations in either group.

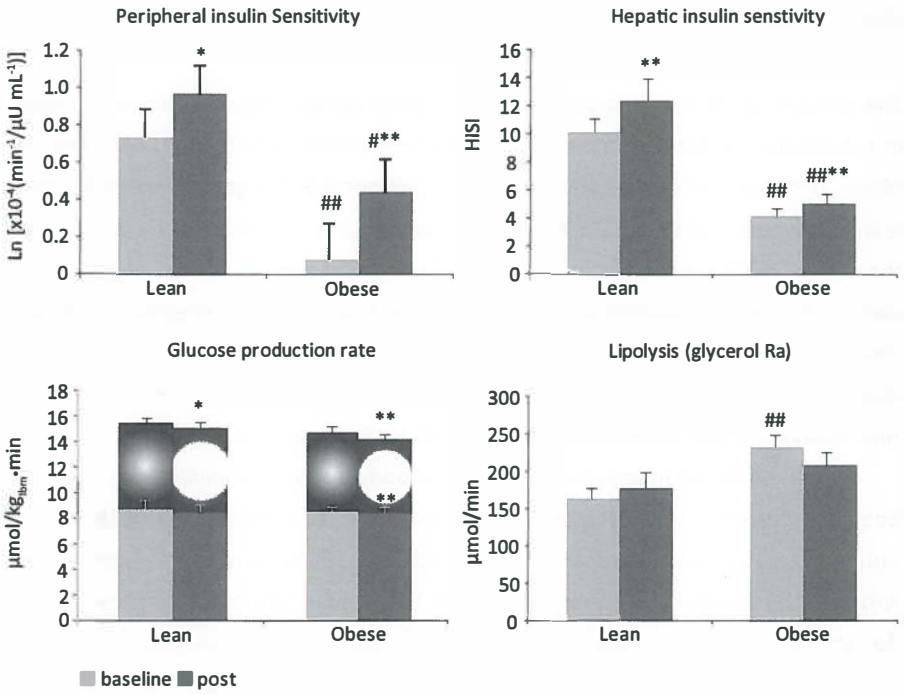

Figure 1. Peripheral Insulin Sensitivity, calculated by the minimal model applied to SLIVGTT data (SI); Hepatic Insulin Sensitivity, measured by Hepatic Insulin Sensitivity Index (HISI); Glucose Production Rate (GPR) consisting of Gluconeogenesis (GNG) (solid part of the bar) and Glycogenolysis (GLY) (upper part of the bar), and Lipolysis (Glycerol Ra) in the lean and obese participants at baseline and post-exercise (mean ± SE).

Significant differences in GPR are depicted above the bars. Significant difference in GLY is depicted inside the bar. Different from baseline within each group: * $p < 0.05$, ** $p < 0.01$. Different between lean and obese participants: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

Interactions between Exercise and Group (Lean and Obese)

There was a significant interaction between the effect of the exercise program and group with respect to insulin and triglyceride concentration ($p = 0.005$ and $p = 0.042$, respectively). All other measured variables had no significant interactions indicating that the response to the exercise program was not different in lean and obese adolescents.

Discussion

The present study demonstrates that a moderate aerobic exercise program resulted in substantial increases in peripheral and hepatic insulin sensitivity in both lean and obese sedentary Hispanic adolescents. The percentage change was not different in lean and obese participants despite significantly lower baseline insulin sensitivity in the obese. Insulin resistance is a major component of obesity and its co-morbidities such as metabolic syndrome and type 2 diabetes. It is, therefore, of great importance, that this program that was of moderate intensity and well accepted by both lean and obese participants (~90% attended sessions at a heart rate corresponding to 85% of that obtained at VO_2 peak) improved fitness as well as insulin sensitivity.

Exercise induced improvements of whole body insulin sensitivity have previously been demonstrated in children and adolescents (9,10,14). However, the methods used, unlabeled oral glucose tolerance test (9), unlabeled euglycemic hyperinsulinemic clamp (10) and the unlabeled frequently sampled intravenous glucose tolerance test (14), do not distinguish peripheral insulin sensitivity (the sensitivity of glucose uptake by peripheral tissue to insulin) and hepatic insulin sensitivity (the sensitivity of hepatic glucose production to insulin). These processes represent different mechanisms for maintenance of normoglycemia. Thus, individual measurements of the two processes would provide new and potentially important information on the physiological effects of exercise on insulin sensitivity. We used the Stable Label Intravenous Glucose Tolerance Test to measure peripheral insulin sensitivity (27), while the Hepatic Insulin Sensitivity Index allowed us to determine hepatic insulin sensitivity in the fasting state (28). This approach enabled us to demonstrate that our aerobic exercise program has significant positive effects both at the site of the liver and peripheral tissue (primarily muscle) in sedentary adolescents. We reported in a previous manuscript (including in part the same participants) (29), that our aerobic exercise program decreased intrahepatic fat content in obese adolescents (primarily those with high hepatic fat content) but did not affect intramyocellular fat in either lean or obese participants. These findings indicate that increased peripheral and hepatic insulin sensitivity are not simply the result of changes in intramyocellular and hepatocellular fat accumulation. Hawley and Lessard (30) recently reviewed various possible mechanisms for exercise induced increase in muscular glucose uptake (peripheral insulin sensitivity) e.g. increased expression of signaling proteins involved in the regulation of glucose uptake and metabolism in skeletal muscle, changes in expression and/or activity of

proteins involved in insulin signaling in the muscle cell and increased lipid turnover and/or oxidation (30). The mechanisms behind exercise effects on hepatic insulin sensitivity are not well understood in humans. However, Heled et al. (31) reported that exercise ameliorated the insulin signaling response and inhibited PEPCK activity in the hepatocyte of diabetes prone fat sand rats. Since it is unethical to perform muscle and liver biopsies in healthy adolescents, we could not explore these avenues in our population. Glucose production, gluconeogenesis and glycogenolysis did not differ between lean and obese participants despite the obese had almost three times higher insulin concentrations, demonstrating their hepatic insulin resistance. In both groups, a small decrease in glucose production was observed in response to the exercise program. This decrease had most likely no clinical relevance in our normoglycemic obese participants with normal glucose tolerance and glucose production rates within the normal range. Thus, a large decrease in glucose production would be unexpected and physiologically unnecessary. The greater increase in hepatic insulin sensitivity than needed for maintenance of normal glucose production might reflect a reserve capacity. If the same effects of an aerobic exercise program would occur in glucose intolerant or diabetic subjects with increased glucose production and resultant increased glucose concentrations, the exercise induced effects on hepatic insulin sensitivity might result in greater effects on glucose production rates with subsequent reduction of glucose concentrations (32). Further research in these populations is needed to address this issue.

We did not observe any effects of exercise on gluconeogenesis. Our method measures total gluconeogenesis, i.e. the contribution from all potential gluconeogenic substrates (glycerol, amino acids and lactate). To our knowledge there are no other reports on the effect of exercise on *total* gluconeogenesis. Bergman et al. (5) showed that in the fed at rest state, gluconeogenesis from lactate, which represents about 10% of glucose production, increased in response to an 8/9 wk aerobic exercise program in lean, sedentary adult males. In contrast, Coggan et al. (6) did not find any effect of a 12 wk aerobic exercise program on fasting at rest gluconeogenesis (from pyruvate) or glycogenolysis.

In our study, the exercise program did not affect fasting lipolysis. Increased lipolysis has been reported after intensive exercise in untrained adult males (7), and in adult male athletes (8). These findings suggest that more intensive aerobic exercise might be needed to affect lipolysis. In agreement with the observations by Wolfe et al. (33),

fasting lipolysis was lower per kg fat mass in our obese participants as compared to their lean counterparts, which might indicate down regulation of lipolysis at the level of the fat cell in obese adolescents (33).

Changes in adiponectin, leptin and hs-CRP concentration did not reach significance as a result of the 12 wk exercise program. Data from other studies on the effects of exercise on these parameters in children, adolescents and adults are inconclusive (9,34-39). Higher adiponectin concentrations in lean as compared to obese subjects have been observed by us and others (21,40). Further, higher adiponectin concentrations have been reported in post-pubertal girls as compared to post-pubertal boys (41). Thus, we believe the larger number of males compared to females in our lean group (4 f/10 m) might explain the lack of baseline difference in adiponectin concentrations in our study. The four lean girls had, in fact, significantly higher adiponectin concentrations than the lean boys as well as the obese girls.

In conclusion, our results demonstrate that a moderate aerobic exercise program that both lean and obese sedentary adolescents could easily comply with resulted in substantial improvements in both peripheral and hepatic insulin sensitivity. Thus, this program could be a useful tool to prevent obesity related illness in Hispanic adolescents. A strength of our study is that a number of potentially confounding factors were controlled; no weight loss; no exercise activity outside the program; a 7-day controlled diet preceding pre- and post-exercise measurements; all participants post-pubertal; strict requirements for attendance and exercise intensity; and all participants sedentary prior to enrolment.

References

1. Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, McDowell M. Physical activity in the United States measured by accelerometer. *Med Sci Sports Exerc* 2008;40:181-8.
2. Manson JE, Nathan DM, Krolewski AS, Stampfer MJ, Willett WC, Hennekens CH. A prospective study of exercise and incidence of diabetes among US male physicians. *JAMA* 1992;268:63-7.
3. Manson JE, Hu FB, Rich-Edwards JW, et al. A prospective study of walking as compared with vigorous exercise in the prevention of coronary heart disease in women. *N Engl J Med* 1999;341:650-8.
4. Tomeo CA, Colditz GA, Willett WC, et al. Harvard Report on Cancer Prevention. Volume 3: prevention of colon cancer in the United States. *Cancer Causes Control* 1999;10:167-80.
5. Bergman BC, Horning MA, Casazza GA, Wolfel EE, Butterfield GE, Brooks GA. Endurance training increases gluconeogenesis during rest and exercise in men. *Am J Physiol Endocrinol Metab* 2000;278:E244-51.
6. Coggan AR, Swanson SC, Mendenhall LA, Habash DL, Kien CL. Effect of endurance training on hepatic glycogenolysis and gluconeogenesis during prolonged exercise in men. *Am J Physiol* 1995;268:E375-83.
7. Phillips SM, Green HJ, Tarnopolsky MA, Heigenhauser GF, Hill RE, Grant SM. Effects of training duration on substrate turnover and oxidation during exercise. *J Appl Physiol* 1996;81:2182-91.
8. Romijn JA, Klein S, Coyle EF, Sidossis LS, Wolfe RR. Strenuous endurance training increases lipolysis and triglyceride-fatty acid cycling at rest. *J Appl Physiol* 1993;75:108-13.
9. Nassis GP, Papantakou K, Skenderi K, et al. Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. *Metabolism* 2005;54:1472-9.
10. Bell LM, Watts K, Siafarikas A, et al. Exercise alone reduces insulin resistance in obese children independently of changes in body composition. *J Clin Endocrinol Metab* 2007;92:4230-5.
11. Gutin B, Owens S. Role of exercise intervention in improving body fat distribution and risk profile in children. *Am J Hum Biol* 1999;11:237-47.
12. Gutin B, Barbeau P, Owens S, et al. Effects of exercise intensity on cardiovascular fitness, total body composition, and visceral adiposity of obese adolescents. *Am J Clin Nutr* 2002;75:818-26.
13. Treuth MS, Hunter GR, Figueroa-Colon R, Goran MI. Effects of strength training on intra-abdominal adipose tissue in obese prepubertal girls. *Med Sci Sports Exerc* 1998;30:1738-43.
14. Shaibi GQ, Cruz ML, Ball GD, et al. Effects of resistance training on insulin sensitivity in overweight Latino adolescent males. *Med Sci Sports Exerc* 2006;38:1208-15.
15. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 2006;295:1549-55.
16. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006;118:1388-93.
17. Narayan KM, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. *JAMA* 2003;290:1884-90.
18. Ischander M, Zaldivar F, Jr., Eliakim A, et al. Physical activity, growth, and inflammatory mediators in BMI-matched female adolescents. *Med Sci Sports Exerc* 2007;39:1131-8.
19. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. *Adv Data* 2000:1-27.
20. Ellis KJ, Abrams SA, Wong WW. Monitoring childhood obesity: assessment of the weight/height index. *Am J Epidemiol* 1999;150:939-46.
21. Sunehag AL, Toffolo G, Campioni M, Bier DM, Haymond MW. Effects of dietary macronutrient intake on insulin sensitivity and secretion and glucose and lipid metabolism in healthy, obese adolescents. *J Clin Endocrinol Metab* 2005;90:4496-502.

22. Sunehag AL, Toffolo G, Treuth MS, et al. Effects of dietary macronutrient content on glucose metabolism in children. *J Clin Endocrinol Metab* 2002;87:5168-78.
23. Treuth MS, Sunehag AL, Trautwein LM, Bier DM, Haymond MW, Butte NF. Metabolic adaptation to high-fat and high-carbohydrate diets in children and adolescents. *Am J Clin Nutr* 2003;77:479-89.
24. Otten J, Pitzi Hellwig J, Meyers L. *Dietary (DRI) Reference Intakes: The Essential Guide to Nutrient Requirements*. The National Academies Press: Washington, DC, 2006.
25. SunehagAL, Treuth MS, Toffolo G, et al. Glucose production, gluconeogenesis, and insulin sensitivity in children and adolescents: an evaluation of their reproducibility. *Pediatr Res* 2001;50:115-23.
26. Chacko SK, Sunehag AL, Sharma S, Sauer PJ, Haymond MW. Measurement of gluconeogenesis using glucose fragments and mass spectrometry after ingestion of deuterium oxide. *J Appl Physiol* 2008;104:944-51.
27. Avogaro A, Bristow JD, Bier DM, Cobelli C, Toffolo G. Stable-label intravenous glucose tolerance test minimal model. *Diabetes* 1989;38:1048-55.
28. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999;22:1462-70.
29. van der Heijden GJ, Wang ZJ, Sauer PJJ, Haymond MW, Rodriguez LM, Sunehag AL. A 12 Week Aerobic Exercise Program Reduces Hepatic Fat Accumulation and Insulin Resistance in Obese, Hispanic Adolescents. *Obesity (Silver Spring) (in press)* 2009.
30. Hawley JA, Lessard SJ. Exercise training-induced improvements in insulin action. *Acta Physiol (Oxf)* 2008;192:127-35.
31. Heled Y, Shapiro Y, Shani Y, et al. Physical exercise enhances hepatic insulin signaling and inhibits phosphoenolpyruvate carboxykinase activity in diabetes-prone Psammomys obesus. *Metabolism* 2004;53:836-41.
32. Gastaldelli A, Baldi S, Pettiti M, et al. Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans: a quantitative study. *Diabetes* 2000;49:1367-73.
33. Wolfe RR, Peters EJ, Klein S, Holland OB, Rosenblatt J, Gary H, Jr. Effect of short-term fasting on lipolytic responsiveness in normal and obese human subjects. *Am J Physiol* 1987;252:E189-96.
34. Kim ES, Im JA, Kim KC, et al. Improved insulin sensitivity and adiponectin level after exercise training in obese Korean youth. *Obesity (Silver Spring)* 2007;15:3023-30.
35. Jones TE, Basilio JL, Brophy PM, McCammon MR, Hickner RC. Long-term Exercise Training in Overweight Adolescents Improves Plasma Peptide YY and Resistin. *Obesity (Silver Spring)* 2009.
36. Gutin B, Ramsey L, Barbeau P, et al. Plasma leptin concentrations in obese children: changes during 4-mo periods with and without physical training. *Am J Clin Nutr* 1999;69:388-94.
37. Simpson KA, Singh MA. Effects of exercise on adiponectin: a systematic review. *Obesity (Silver Spring)* 2008;16:241-56.
38. Bouassida A, Chamari K, Zaouali M, Feki Y, Zbidi A, Tabka Z. Review on leptin and adiponectin responses and adaptations to acute and chronic exercise. *Br J Sports Med* 2008.
39. Puglisi MJ, Fernandez ML. Modulation of C-reactive protein, tumor necrosis factor-alpha, and adiponectin by diet, exercise, and weight loss. *J Nutr* 2008;138:2293-6.
40. Weiss R, Dziura J, Burgert TS, et al. Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med* 2004;350:2362-74.
41. Bottner A, Kratzsch J, Muller G, et al. Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. *J Clin Endocrinol Metab* 2004;89:4053-61.

Chapter 4

Twelve weeks moderate aerobic exercise without dietary intervention or weight loss does not affect 24 h energy expenditure in lean and obese adolescents

Gert-Jan van der Heijden

Pieter J.J. Sauer

Agneta L. Sunehag

Am J Clin Nutr (Accepted for publication)

Abstract

Background: Exercise might have a persistent effect on energy expenditure and fat oxidation resulting in increased fat loss. However, even without weight loss exercise results in positive metabolic effects. The impact of an aerobic exercise program on 24 h total energy expenditure (TEE) and its components, basal (BEE), sleep (SEE) and awake sedentary (SEDEE) energy expenditure and substrate oxidation has not been studied in lean and obese adolescents.

Objective: To test the hypothesis that 24 h energy expenditure and fat oxidation are increased in lean and obese adolescents after 12 wks moderate aerobic exercise without dietary interventions and weight loss.

Participants and Design: Twenty-eight post-pubertal Hispanic adolescents (13 lean: 15.3 ± 0.3y; 20.2 ± 0.7kg/m^2; 18.7 ± 1.6% body fat and 15 obese: 15.6 ± 0.3y; 33.1 ± 0.9kg/m^2; 38.1 ± 1.4% body fat) (mean±SE), completed a 12 wk aerobic exercise program (4x30 min/wk at ≥ 70% of VO_2 peak) without weight loss. Energy expenditure and substrate oxidation were quantified by 24 h room-calorimetry at baseline and post-exercise.

Results: This aerobic exercise program did not affect 24 h TEE, BEE, SEE and SEDEE in lean and obese participants. In obese adolescents, RQ (respiratory quotient) and substrate oxidation did also not change. In lean adolescents, 24 h RQ, and RQ during SEE decreased (both $p < 0.01$) and fat oxidation increased ($p < 0.01$).

Conclusions: A 12 wk aerobic exercise program did not increase TEE, BEE, SEE and SEDEE in either lean or obese sedentary adolescents. Further, 24 h fat oxidation did not change in obese while it increased in lean adolescents.

Introduction

Efficient and well accepted strategies to prevent or delay obesity related morbidity in children and adolescents are much needed, particularly in minority groups (1-3). Most studies on the outcome of interventions include both exercise and weight loss. Since weight loss is difficult to achieve and maintain in adolescents, we wanted to determine whether an exercise program alone (i.e. without weight loss) would be well accepted by sedentary lean and obese Hispanic adolescents, and whether it would result in reduced insulin resistance and fat deposits associated with obesity related disorders (e.g. visceral and hepatic fat content). We recently reported that a moderate 12 wk aerobic exercise program without diet interventions and weight loss, increased hepatic and peripheral insulin sensitivity in both lean and obese participants (4). In addition, the program decreased visceral and hepatic fat content in the obese adolescents (5). An important part of the study was to determine whether any of the components of 24 h total energy expenditure (TEE) [basal (BEE), sleep (SEE) and awake sedentary (SEDEE)] and/or substrate oxidation rates are affected by exercise itself (i.e. without weight loss) and if so if these changes are related to any specific metabolic and/or fat distribution improvements.

Numerous studies have addressed energy expenditure and substrate oxidation (fat oxidation in particular) during and after individual exercise bouts and in relation to various exercise programs. Some studies have demonstrated increased resting energy expenditure in response to exercise (6-9), while others have not found any effects (10). These differences might be explained by type, duration and intensity of the exercise and whether lean body mass increased or not. It has been shown that energy expenditure can remain increased for 36-48 h after an exercise bout (11). Thus, the timing of measurements in relation to the exercise session might also be of importance (11). Similarly, the effect of exercise on fat oxidation has been shown to depend on intensity, duration, and age and body composition of the participants. In this regard, it has been reported that low to moderate intensity exercise increases fat oxidation more than high intensity aerobic training (12,13); the maximal rate of fat oxidation occurs at a lower percentage of VO_2 peak in post-pubertal as compared to pubertal and pre-pubertal children (14,15); training increased resting fat oxidation in lean adults (16-18), while its influence on resting fat oxidation in overweight and obese adults was inconclusive (6,7,19-21). In general, the referenced studies indicate the potential of exercise to increase energy expenditure and fat oxidation. Therefore, we hypothesized

that our 12 wk moderate exercise program would increase TEE, BEE, SEE and SEDEE and increase fat oxidation.

To our knowledge there are no published reports on 24 h TEE and its components, BEE, SEE and SEDEE and substrate oxidation in adolescents in response to an exercise program. In fact, most studies also in adults measured energy expenditure and/or substrate oxidation using indirect calorimetry over shorter periods of time (7,8,10,12, 21-24). Thus, the present study has the potential to address several of these gaps in our knowledge.

Participants and Methods

Participants

After approval of the protocol by the Baylor College of Medicine Institutional Review Board for Human Subject Research, and the General Clinical Research Center Advisory Board, obese and lean adolescents were recruited by local advertisement. Adolescents were screened and enrolled in the study after written assent from the participant and consent from the legal guardian were obtained.

The calorimeter measurements were part of an extensive study on the metabolic effects of aerobic exercise (4,5). Twenty-eight (28) post pubertal Hispanic adolescents (Tanner IV – V), 13 lean and 15 obese, were studied **(Table 1)**. The lean participants had body mass index (BMI) < 85th and the obese BMI > 95th percentile for age according to CDC growth charts (25). However, BMI might not be an optimal marker of leanness/ obesity (26). Thus, to assure that our lean participants were indeed lean not only with regard to BMI criteria but also to fatness, they must have < 27% body fat as measured by dual-energy x-ray absorptiometry (DXA) **(Table 1)**. Participants had been lean or obese for ≥ 5 yrs and reported stable body weight for at least 6 mo. Only sedentary adolescents were included, i.e. they did not participate in any school or after school organized athletic activities and performed < 45 min light to moderate physical activity/ wk.

All participants were Hispanic (parents and grandparents of Hispanic descent by self report). They were in good health as determined by a medical history, a physical examination and a standard blood chemistry analysis including blood lipids, liver- and kidney function tests, hemoglobin, hematocrit, hemoglobin A1c and fasting and 2 h post-prandial glucose response. Participants were not taking medications including

birth control pills and had no first-degree relatives with diabetes. Adolescents with morbid obesity (body fat % > 50, sleep apnea, Pickwick syndrome or cor pulmonale) were excluded.*Study Design*

Each participant was studied on two separate occasions: 1) The weekend before start of the exercise program (baseline), 2) The weekend after the final exercise session of the 12 wk program (post-exercise). All procedures were identical on both study occasions.

The participants were admitted to the Metabolic Research Unit (MRU) at the Children's Nutrition Research Center, Houston, where 24 h calorimeter measurements were performed to assess energy expenditure and substrate oxidation rates (27-29).

To exclude effects of dietary intake on measurements obtained at baseline and post-exercise, each participant received, prior to both studies, an identical 7 d low-carbohydrate (CHO)/high-fat diet (30% CHO, 55% fat, and 15% protein; 20% of the total CHO content as fructose) (27-29). Total energy intake was calculated to correspond to each individual's requirement according to the Institute of Medicine Dietary Reference Intakes (30). The food was delivered to the participants' homes by the metabolic research kitchen. Non-consumed food was returned and examined for constituents and the energy and macronutrient content of the consumed food was calculated by difference (27-29). All meals served during the calorimeter study had an identical composition to that of the 7 d standardized diet consumed at home prior to the baseline and post-exercise studies, except that total energy intake was reduced by about 5% to adjust for a slightly lower activity level in the calorimeter. In order to determine the effect of exercise alone, participants were told not to make lifestyle changes and keep to their habitual diet except adhere to the controlled diet provided the week prior to both study occasions.

Exercise Program

For the duration of 12 wks, participants came to the physical therapy unit at Texas Children's Hospital twice a week for a 30 min aerobic exercise session on a treadmill, elliptical or bicycle (dependent on the preference of the participant). Each exercise session was preceded and followed by 10 min of warm up/cool down and stretching. The exercise intensity level was designed to result in a heart rate corresponding to at least 70% of that obtained at $VO_{2\ peak}$ at baseline (see cardiovascular fitness section), which corresponded to heart rates >140 beats/min in all participants. This rate had to be maintained for the entire 30 min session. Experienced exercise physiologists

were responsible for the training sessions together with the principal investigator. Participants were instructed to perform a similar program (same duration and intensity) twice a week at home, i.e., a total of 4 exercise sessions per week. To assure that the desired heart rate (exercise intensity) was achieved and maintained for 30 min, each participant wore a heart rate monitor, Polar S-710 (Health Check Systems, Brooklyn, NY) during all home and hospital exercise sessions. Information from the monitors was downloaded and discussed with the participants on a weekly basis. In order to measure the effect of the exercise program alone, participants were asked not to perform any exercise outside the program. Their weight was assessed twice a week in conjunction with the exercise sessions to assure weight stability.

The last exercise session took place at least 24 h prior to start of the calorimeter study and approximately 38 h prior to the measurement of BEE.

The compliance with the program was very good as demonstrated by 88 ± 2 and 91 ± 2% attendance in the lean and obese participants, respectively, at heart rates corresponding to 85 ± 1 (lean) and 85 ± 2 (obese) % of that obtained at VO_{2peak}.

Cardiovascular Fitness

Peak oxygen consumption ($VO_{2\ peak}$) was measured at baseline and post-exercise using a modified Bruce treadmill protocol. The treadmill test started at a speed of 1.7 mph. Subsequently, the speed and incline were gradually increased every 3 min until maximal exercise capacity of the participant was reached. Oxygen consumption was measured with a Vmax-229 metabolic cart (Sensormedics, Anaheim, CA). $VO_{2\ peak}$ was determined using standard criteria, specifically a heart rate > 195 beats/min or a respiratory quotient (RQ) > 1.0 at peak exercise (29).

Room Respiration Calorimetry

Oxygen consumption (VO_2), carbon dioxide production (VCO_2), and RQ, defined as VCO_2/VO_2, were measured continuously in 17 m^3 room calorimeters for 24 h at baseline and post-exercise. The design and performance of the respiration calorimeters were described in detail previously (31). Mean (± SD) errors from 24 h infusions of nitrogen and carbon dioxide were -0.34 ±1.24% for VO_2 and 0.11 ± 0.98% for VCO_2 (31). The rooms are equipped with a bed, desk, chair, lamp, toilet, sink, television and VCR, video games, and telephone. Heart rate was recorded by telemetry (DS-3000; Fukuda Denshi, Tokyo), and physical activity, expressed as activity counts/ min, was monitored with a Doppler microwave sensor (D9/50; Microwave Sensors, Ann Arbor, MI). All urine was collected

and pooled during the 24 h calorimeter test. For data analysis, we assumed that urinary nitrogen excretion was constant over the 24 h period. Urine samples were digested using sulfuric acid (modified Kjeldahl) and the nitrogen concentration was determined spectrophotometrically using a Technicon AAII colorimeter (Technicon Industrial Systems, Tarrytown, NY). From the 24 h VO_2, VCO_2, and urinary nitrogen excretion, total energy expenditure and its components were calculated using the Weir equation (32) and Non Protein Respiratory Quotient (NPRQ) and substrate oxidation according to Frayn (33). Energy balance was computed as total energy intake (TEI) kcal/d minus TEE kcal/d. The calorimeter test began at 1600. While in the calorimeter, the participants were allowed free choice of sedentary activities (television, VCR, Nintendo, arts and crafts, reading, etc). Four meals were consumed in the calorimeter. Day 1: dinner at 1730 h and a snack at 1830 h (no food was allowed after 1900). Day 2: breakfast at 0830 h and lunch at 1200 h. Bedtime was at 2100-2200 h.

Components of 24 h Total Energy Expenditure

Total energy expenditure (TEE): The total energy expenditure over 24 h.

Basal energy expenditure (BEE): BEE was measured after a 12 h overnight fast. The participants were awakened at 0630 and asked to void, after which they returned to sleep. The participants were again awakened ~30 min later, and after confirmed awake, their basal energy expenditure was measured for 40 min, beginning at 0720 h. The participants were monitored both visually and by an activity sensor to confirm that they were lying still (< 50 activity counts/min) for the entire measurement.

Sleep energy expenditure (SEE): The mean energy expenditure during all night time sleeping, as verified by a heart rate monitor and motion sensor.

Sedentary awake energy expenditure (SEDEE): Energy expenditure after termination of the BEE measurement (0800 h) until the end of the calorimeter study (1600 h). Only sedentary activity was allowed (e.g. television watching).

Statistical Methods

Generalized estimating equations (GEE) (SPSS 17.0) were used to assess the effects of the exercise program, weight status (lean or obese) and the interaction between the effect of the exercise program and weight status on TEE, its components (BEE, SEE and SEDEE) and substrate oxidation. Post hoc procedures provided by GEE were used to compare groups at baseline and post-exercise and to assess the effect of the exercise program within each group. This method accounts for repeated measurements in

individuals at baseline and post-exercise and when appropriate for the incorporation of confounding variables including baseline measurements and variables that change with time.

Confounding variables for the between group comparison on energy expenditure and substrate oxidation statistics were sex and activity counts. The confounding variable for within group comparison was activity counts (34). Post hoc analysis per group is reported only when a significant interaction between the effect of the exercise program and weight status was observed. No interaction indicates that there was no difference in the response to exercise in lean and obese participants i.e. a variable might increase, decrease or not change in both groups. In the tables, an asterisk at the post-exercise value in both groups indicates a significant change in both groups. The p-value reflects the combined group analysis.

Results

Participant Characteristics (Table 1)

According to the design, the obese participants had higher body weight, BMI, fat mass and body fat % as compared to the lean participants (all $p < 0.01$). Also lean body mass was higher in obese participants ($p < 0.01$).

The effect of the exercise program on body composition was not different between the lean and obese groups (no significant interaction). Fitness and lean body mass increased to the same extent in both groups (both $p < 0.01$).

Energy Intake in the Calorimeter (Table 2)

In both lean and obese participants, total energy intake (TEI) was not different at baseline and post-exercise. TEI was higher in obese participants as a result of their higher requirements as calculated by Dietary Reference Intakes (30) ($p < 0.05$). In addition, the macronutrient distribution of the intake corresponded to the design and was not different between groups and study occasions.

Energy Expenditure (Tables 2 and 3)

At baseline, TEE (kcal/d) and its components BEE, SEE and SEDEE (kcal/min), were significantly higher in the obese group as compared to the lean (all $p < 0.01$). These differences remained significant (all $p < 0.01$) when adjusted for confounding variables

(sex and activity counts). TEE expressed as kcal/kg·d was lower in obese as compared to lean adolescents, whereas TEE expressed as kg/lean body mass did not differ.

Table 1. Participant characteristics

	Lean Participants		Obese Participants		Interaction[$] (p-value)
	Baseline	Post Exercise	Baseline	Post Exercise	
N	13		15		
Male/Female	9/4		7/8		
Age	15.2 ± 0.3		15.6 ± 0.3		
Weight (kg)	56.2 ± 2.7	56.8 ± 2.8	89.8 ± 3.2##	89.4 ± 3.1##	0.100
BMI (kg/m²)	20.2 ± 0.7	20.3 ± 0.7	33.1 ± 0.9##	32.9 ± 0.8##	0.267
Lean body mass (kg)	44.0 ± 2.3	45.2 ± 2.5**	53.6 ± 2.8##	54.6 ± 2.9#**	0.682
Fat mass (kg)	10.5 ± 1.0	10.4 ± 1.0	34.0 ± 1.4##	33.1 ± 1.4##	0.126
Bodyfat %	18.7 ± 1.6	18.2 ± 1.6	38.1 ± 1.4##	37.0 ± 1.5##	0.345
$VO_{2\ peak}$ (L/min)	2.15 ± 0.15	2.48 ± 0.12**	2.49 ± 0.15	2.76 ± 0.14**	0.916

Data are presented as mean ± SE

Different between lean and obese participants # p < 0.05, ## p < 0.01

Different from baseline within each group: * p < 0.05, ** p < 0.01

General Estimating Equations (GEE)[$] were used to determine potential interaction between lean and obese participants with regard to effect of exercise. When a significant interaction was found between the lean and obese group, post hoc procedures provided by GEE were used to compare groups at baseline and post-exercise and to assess the effect of the exercise program within each group. When there was no interaction (i.e. the response to exercise did not differ between the groups) but both groups showed a decrease or increase in the measured variable, combined statistics were used to determine the significance level.

* p < 0.05, ** p < 0.01

The effect of the exercise program on energy expenditure was not different between lean and obese participants. No significant change in TEE (kcal/d and kcal/kg_{lbm}·d), BEE, SEE and SEDEE was observed after the exercise program in either group. The change in TEE (kcal/kg·d) in lean participants was no longer significant after adjustment for activity counts.

Energy expenditure values obtained at 1 h intervals for the duration of the calorimeter measurements (baseline and post-exercise) demonstrate higher energy expenditure (kcal/min) in obese as compared to lean participants at all time points with no effect of the exercise program (Figure 1). Energy balance was positive in both groups on both study occasions. We did not measure fecal losses. These losses would most likely cancel out the slightly positive energy balance.

Table 2. 24 h energy intake and expenditure in the calorimeter

	Lean participants (n = 13)		Obese Participants (n = 15)		Interaction[$] (p-value)
	Baseline	Post Exercise	Baseline	Post Exercise	
TEI (kcal/d)	2134 ± 85	2134 ± 85	2524 ± 142[#]	2506 ± 148[#]	0.306
TEE (kcal/d)	1918 ± 86	1860 ± 73	2332 ± 106[##]	2341 ± 97[##]	0.508
Energy balance	215 ± 50	274 ± 58	192 ± 89	164 ± 83	0.141
TEE (kcal/kg·d)	34 ± 1	33 ± 1[**]	26 ± 1[##]	26 ± 2[##]	0.035
TEE (kcal/kg_{lbm}·d)	44 ± 1	42 ± 1	43 ± 1	43 ± 1	0.178
Activity counts (counts/min)	104 ± 7	84 ± 6[*]	93 ± 8	86 ± 8[*]	0.247
24 h RQ	0.84 ± 0.01	0.81 ± 0.01[**]	0.81 ± 0.01[##]	0.82 ± 0.01	0.006
24 h NPRQ	0.84 ± 0.01	0.81 ± 0.01[**]	0.81 ± 0.01[##]	0.82 ± 0.01	0.009
24 h Substrate Intake					
Carbohydrate (kcal/d)	652 ± 26	652 ± 26	776 ± 45[#]	763 ± 48[#]	0.080
(% TEI)	31 ± 1	31 ± 1	31 ± 1	30 ± 1	0.105
Fat (kcal/d)	1151 ± 49	1151 ± 49	1359 ± 79[#]	1354 ± 81[#]	0.678
(% TEI)	54 ± 1	54 ± 1	54 ± 1	54 ± 1	0.320
Protein (kcal/d)	331 ± 14	331 ± 14	389 ± 20[#]	388 ± 20[#]	0.345
(% TEI)	15 ± 1	15 ± 1	15 ± 1	16 ± 1	0.305
24 h substrate Oxidation					
Carbohydrate (kcal/d)	717 ± 40	557 ± 39[**]	694 ± 55	750 ± 68[#]	0.009
(% TEE)	37 ± 1	30 ± 2[**]	30 ± 2[##]	32 ± 2	0.003
Fat (kcal/d)	886 ± 50	979 ± 51	1313 ±74[##]	1263 ± 88[##]	0.208
(% TEE)	46 ± 1	52 ± 2[**]	57 ± 2[##]	54 ± 3	0.034
Protein (kcal/d)	312 ± 26	338 ± 22	340 ± 32	335 ± 32	0.325
(% TEE)	16 ± 1	18 ± 1	14 ± 1	14 ± 1	0.129

Total Energy Intake (TEI), Total Energy Expenditure (TEE), Percentage of Total Energy Intake (% TEI), Percentage of Total Energy Expenditure (% TEE), Respiratory Quotient (RQ), Non Protein Respiratory quotient (NPRQ)

Data are presented as mean ± SE

Different between lean and obese participants [#] $p < 0.05$, [##] $p < 0.01$

Different from baseline within each group: * $p < 0.05$, ** $p < 0.01$

General Estimating Equations (GEE)[$] were used to determine potential interaction between lean and obese participants with regard to effect of exercise. When a significant interaction was found between the lean and obese group, post hoc procedures provided by GEE were used to compare groups at baseline and post-exercise and to assess the effect of the exercise program within each group. When there was no interaction (i.e. the response to exercise did not differ between the groups) but both groups showed a decrease or increase in the measured variable, combined statistics were used to determine the significance level.

* $p < 0.05$, ** $p < 0.01$

Data in the table are not corrected for activity counts and sex

Substrate Oxidation (Tables 2 and 3)

At baseline, 24 h total RQ and NPRQ, as well as basal, sleep and sedentary awake RQ were significantly lower in obese as compared to lean participants ($p < 0.01$, $p < 0.01$, $p < 0.01$, $p < 0.01$ and $p < 0.05$, respectively). Fat oxidation expressed per kcal/d and as % of total energy expenditure (% TEE) was higher in obese as compared to lean participants (both $p < 0$ .01). Total carbohydrate oxidation (kcal/d) was not different between groups, while carbohydrate oxidation (% TEE) was significantly lower in obese participants ($p < 0.01$). Adjusted for confounding variables (sex and activity counts), all differences remained significant. Protein oxidation (kcal/d and % TEE) was not different between groups.

Table 3. Components of 24 h energy expenditure and substrate oxidation

		Lean Participants (n = 13)		Obese Participants (n = 15)		Interaction$^{\$}$ (p-value)
		Baseline	Post Exercise	Baseline	Post Exercise	
Total 24 h	EE (kcal/min)	1.33 ± 0.06	1.29 ± 0.05	1.62 ± 0.07$^{\#\#}$	1.63 ± 0.07$^{\#\#}$	0.517
BEE	EE (kcal/min)	1.01 ± 0.03	1.03 ± 0.04	1.23 ± 0.06$^{\#\#}$	1.22 ± 0.06$^{\#\#}$	0.397
	RQ	0.81 ± 0.01	0.77 ± 0.01	0.77 ± 0.02$^{\#}$	0.77 ± 0.02	0.072
SEE	EE (kcal/min)	0.96 ± 0.03	0.95 ± 0.04	1.18 ± 0.05$^{\#\#}$	1.17 ± 0.05$^{\#\#}$	0.957
	RQ	0.83 ± 0.01	0.80 ± 0.01**	0.79 ± 0.01$^{\#\#}$	0.80 ± 0.01	0.044
SEDEE	EE (kcal/min)	1.49 ± 0.08	1.44 ± 0.06	1.81 ± 0.09$^{\#\#}$	1.80 ± 0.08$^{\#\#}$	0.469
	RQ	0.83 ± 0.01	0.81 ± 0.01	0.81 ± 0.01$^{\#}$	0.81 ± 0.01	0.052

Basal Energy Expenditure (BEE), Sleep Energy Expenditure (SEE), Awake Sedentary Energy Expenditure (SEDEE) Respiratory quotient (RQ).

Data are presented as mean ± SE

Different between lean and obese participants $^{\#}p < 0.05$, $^{\#\#}p < 0.01$

Different from baseline within each group: * $p < 0.05$, ** $p < 0.01$

General Estimating Equations (GEE)$^{\$}$ were used to determine potential interaction between lean and obese participants with regard to effect of exercise. When a significant interaction was found between the lean and obese group, post hoc procedures provided by GEE were used to compare groups at baseline and post-exercise and to assess the effect of the exercise program within each group. When there was no interaction (i.e. the response to exercise did not differ between the groups) but both groups showed a decrease or increase in the measured variable, combined statistics were used to determine the significance level. * $p < 0.05$, ** $p < 0.01$

Data in the table are not corrected for activity counts and sex

In **obese participants**, the exercise program did not affect 24 h RQ and NPRQ, or basal, sleep and sedentary awake RQ. In addition, 24 h total carbohydrate, fat and protein oxidation (kcal/d and % TEE) did not change as a result of the exercise program.

In contrast, in **lean participants**, the exercise program resulted in a significant decrease in 24 h total RQ and NPRQ, and sleep RQ (all $p<0.01$). Fat oxidation (% TEE) increased ($p < 0.01$) after the exercise program, while carbohydrate oxidation (kcal/d and % TEE) decreased (both $p < 0.01$). Thus, post-exercise, carbohydrate and fat oxidation rates were no longer different in obese and lean participants. Protein oxidation (kcal/d and % TEE) remained unchanged. All differences remained significant corrected for activity counts. RQ values at 1 h intervals throughout the calorimeter study are shown for both groups in **Figure 2**. The figure shows an overall pattern of lower RQ in obese as compared to lean participants at baseline and a decrease in RQ post exercise in lean participants.

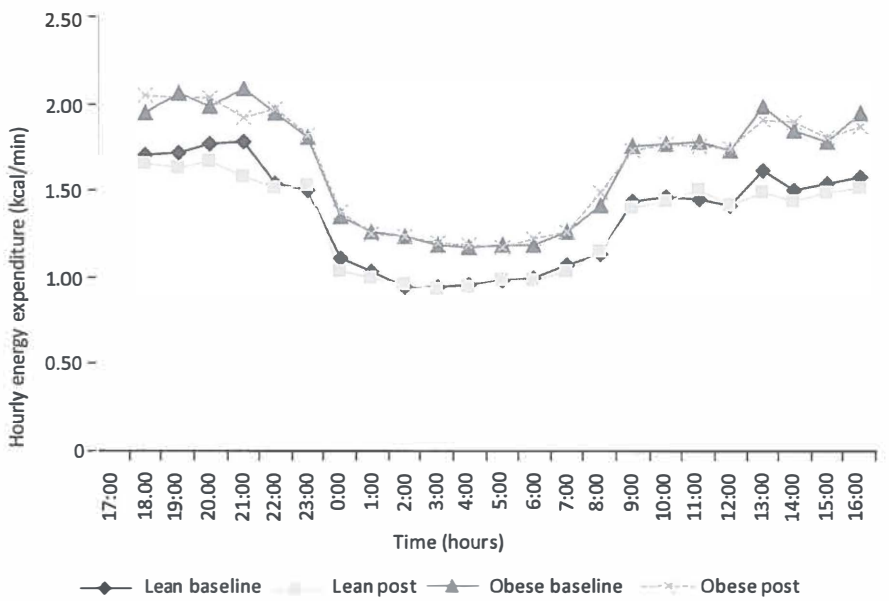

Figure 1. Hourly energy expenditure (EE) (kcal/min) in lean and obese participants during the course of the calorimeter study at baseline and post exercise.

Discussion

The results of the present 24 h calorimeter measurements show that a 12 wk moderate aerobic exercise program without weight loss did not affect TEE or its components: BEE, SEE and SEDEE in either lean or obese adolescents. No changes in substrate oxidation were observed in obese adolescents. In lean adolescents, carbohydrate oxidation decreased and fat oxidation increased in response to the exercise program.

Obese adolescents had significantly higher energy expenditure compared to their lean counterparts. It has been suggested that the metabolically active lean body mass is the main predictor of energy expenditure (34-36). Thus, the higher TEE observed in the obese participants in our study can be explained by their higher lean body mass. This is further supported by the fact that TEE expressed per kg lean body mass was not different in lean and obese participants. TEE expressed per kg bodyweight was however, lower in obese participants, indicating that the large fat mass has lesser effect on energy metabolism than lean body mass.

We found no effect of our exercise program on 24 h TEE. Reports from other investigators are divergent. Some studies have demonstrated increased resting energy expenditure in response to exercise (6-9), while others have not found any effects (10). Several factors might explain these inconsistencies. Most studies measured resting energy expenditure (REE) over a short, 30 min period (7,8,10). Extrapolating these data to a 24 h period might provide erroneous estimates of 24 h total energy expenditure. The use of 24 h room calorimetry allowed us to accurately measure total energy expenditure taking into account the variation resulting from basal, sleep and sedentary activity (31). Another potential confounder is the timing of the measurements in relation to the previous exercise session (6-10). Although the acute effect of an exercise bout on energy expenditure is greatest during the first couple of hours, some effects might persist for 36-48 h depending on duration and intensity of the exercise (11). In the present study, post-exercise TEE was measured between 24 and 48 h after the last exercise session and BEE at least 38 h after the final exercise session. In addition, energy expenditure was not different during the first and the last hours of the 24 h measurement period in either lean or obese participants (**Figure 2**). Therefore, it is highly unlikely that our energy expenditure data are overestimated. Two studies (6,7) measured energy expenditure at 48 and 72 h, respectively, after the final exercise session of an exercise program (i.e. persistent acute exercise effects were unlikely). The investigators reported that 6 mo 3 x wk resistance training (6) and 6 mo 3-5 x wk 20-45 min/ session aerobic exercise at 60-

75 % of heart rate reserve (7) increased energy expenditure. They concluded that their results were primarily related to increased lean body mass but suggested that exercise induced changes in other parameters known to affect basal energy expenditure e.g. sympathetic nervous system activity (37) and thyroid hormones (38) also might have played a role. The small increase in lean body mass (2-3%) in our participants might have been insufficient to affect energy expenditure. The intensity and duration of an exercise program required to modulating hormone and sympathetic nervous system activity in adolescents is not known.

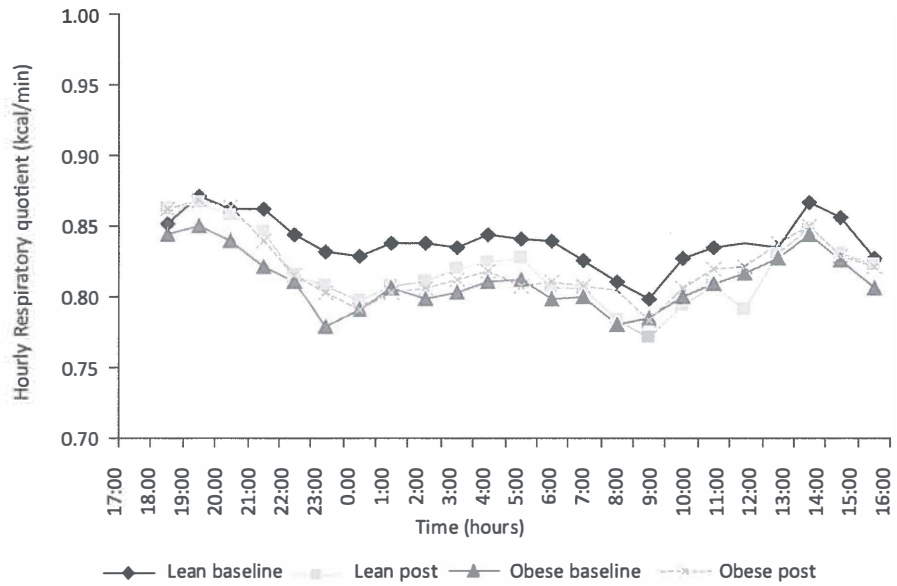

Figure 2. Hourly respiratory quotients (RQ's) in lean and obese participants during the course of the calorimeter study at baseline and post exercise

We have previously shown that substrate oxidation rates are affected by the macronutrient composition of the dietary intake (29). Therefore, energy content and macronutrient distribution of the intake were well controlled and did not differ on the two study occasions (baseline and post-exercise). This allowed us to accurately assess the effect of the exercise program on substrate oxidation and compare the data between lean and obese adolescents.

At baseline, total fat oxidation (kcal/d) was significantly higher in obese as compared to lean participants. In addition, obese participants relied to a greater extend on fat oxidation for total energy expenditure than the lean, even though fat oxidation was the principal contributor to energy expenditure in both groups (57 and 46%, respectively). This is in agreement with the reports from Maffeis et al. (39). These investigators demonstrated that total fat oxidation was highly related to fat mass and that the rate of exogenous fat oxidation increased with adiposity. They postulated that increased fat oxidation might be a protective measure to avoid a further increase in fat mass in obese children (39). In agreement with the observation by Rueda Maza et al. (40), total carbohydrate oxidation (kcal/d) was not different between our lean and obese participants. However, the proportion of total energy expenditure derived from carbohydrates was lower in obese as compared to lean adolescents.

The exercise program did not change substrate oxidation in the obese participants. On the other hand, fat oxidation (% TEE) increased and carbohydrate oxidation (kcal/d and % TEE) decreased in the lean participants. Similar findings have been reported in adults (7,16-18,20). Physical activity increased resting fat oxidation in lean adults (16-18), while no increase was observed in overweight and obese adults (7,20). A recently published study (6) found increased resting and sleep fat oxidation after 6 mo of low volume resistance training in overweight adults as compared to a non exercising control group. However, 24 h fat oxidation remained unchanged (6). The explanation for this discrepancy between lean and obese individuals is not well understood. Blaak et al. (19) proposed that obese individuals have impaired capacity to oxidize fat possibly prohibiting an increase in fat oxidation in response to exercise. At baseline, fat oxidation was higher in our obese as compared to our lean participants, which would contradict impaired fat oxidation. However, the lean but not the obese adolescents responded with increased fat oxidation to the exercise program. This might indicate impaired metabolic flexibility in obese adolescents. Rueda-Maza et al. (40) suggested that lower carbohydrate oxidation in obese as compared to lean children would indicate reduced glycogen turnover in the obese. We measured glycogenolysis in our adolescents and found no difference between lean and obese participants at baseline despite lower carbohydrate oxidation in the obese participants (4). Post exercise, carbohydrate oxidation rates were similar in obese and lean adolescents despite glycogenolysis decreased in obese but remained unchanged in lean participants (4). These findings contradict a relationship between glycogen turnover and carbohydrate oxidation rates.

The calorimeter test is an important part of our extensive study of the metabolic effect of a 12 wk aerobic exercise program. The purpose of this study was to determine the effect of a controlled aerobic exercise program alone i.e. **without weight loss** on energy expenditure and its sub components, substrate oxidation, body composition, detailed body fat distribution, insulin sensitivity, and glucose and lipid metabolism. We have previously reported (in partly the same participants) (4,5) that this moderate 12 wk aerobic exercise program resulted in increased peripheral insulin sensitivity, 59 ± 19 and 35 ± 14 % in lean and obese participants, respectively, and hepatic insulin sensitivity, 23 ± 4 and 19 ± 7 %, respectively. In addition the program decreased visceral and hepatic fat content in obese adolescents. Collectively, our data show that in obese adolescents, important reductions in visceral and hepatic fat and significant improvements in both peripheral and hepatic insulin sensitivity occurred without weight loss and without changes in whole body TEE and its components or in substrate oxidation rates. These findings might indicate that aerobic exercise has its major effects on the cellular level in muscle (41) and liver (42) not reflected by changes in whole body energy expenditure and substrate oxidation. Since it is unethical to perform muscle and liver biopsies in healthy adolescents, these avenues could not be pursued. We speculate that an exercise program with greater effect on lean body mass and/or a combined exercise and weight loss intervention would be required to achieve significant increase in energy expenditure and fat oxidation.

In conclusion, a 12 wk moderate aerobic exercise program did not increase TEE, BEE, SEE and SEDEE in either obese or lean adolescents. In lean participants fat oxidation increased post exercise, while in the obese, oxidation rates were not affected. Thus, in obese adolescents, changes in whole body energy expenditure and substrate oxidation rates are not required to reduce visceral and hepatic fat content and improve peripheral and hepatic insulin sensitivity.

References

1. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006;118:1388-93.
2. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 2006;295:1549-55.
3. Narayan KM, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. *JAMA* 2003;290:1884-90.
4. van der Heijden G, Toffolo G, Manesso E, Sauer PJJ, Sunehag AL. Aerobic Exercise Increases Peripheral and Hepatic Insulin Sensitivity in Sedentary Adolescents. *J Clin Endocrinol Metab (E-pub ahead of print)* 2009 PMID 19808855.
5. van der Heijden GJ, Wang ZJ, Sauer PJJ, Haymond MW, Rodriguez LM, Sunehag AL. A 12 Week Aerobic Exercise Program Reduces Hepatic Fat Accumulation and Insulin Resistance in Obese, Hispanic Adolescents. *Obesity (Silver Spring) (E-pub ahead of print)* 2009 PMID 19696755.
6. Kirk EP, Donnelly JE, Smith BK, et al. Minimal Resistance Training Improves Daily Energy Expenditure and Fat Oxidation. *Med Sci Sports Exerc* 2009;41:1122-9.
7. Potteiger JA, Kirk EP, Jacobsen DJ, Donnelly JE. Changes in resting metabolic rate and substrate oxidation after 16 months of exercise training in overweight adults. *Int J Sport Nutr Exerc Metab* 2008;18:79-95.
8. Byrne HK, Wilmore JH. The effects of a 20-week exercise training program on resting metabolic rate in previously sedentary, moderately obese women. *Int J Sport Nutr Exerc Metab* 2001;11:15-31.
9. Goran MI, Calles-Escandon J, Poehlman ET, O'Connell M, Danforth E, Jr. Effects of increased energy intake and/or physical activity on energy expenditure in young healthy men. *J Appl Physiol* 1994;77:336-72.
10. Byrne HK, Wilmore JH. The relationship of mode and intensity of training on resting metabolic rate in women. *Int J Sport Nutr Exerc Metab* 2001;11:1-14.
11. Speakman JR, Selman C. Physical activity and resting metabolic rate. *Proc Nutr Soc* 2003;62:621-34.
12. Venables MC, Achten J, Jeukendrup AE. Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *J Appl Physiol* 2005;98:160-7.
13. Romijn JA, Coyle EF, Sidossis LS, et al. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol* 1993;265:E380-91.
14. Riddell MC. The endocrine response and substrate utilization during exercise in children and adolescents. *J Appl Physiol* 2008;105:725-33.
15. Zunquin G, Theunynck D, Sesboue B, Arhan P, Bougle D. Evolution of fat oxidation during exercise in obese pubertal boys: clinical implications. *J Sports Sci* 2009;27:315-8.
16. Phillips SM, Green HJ, Tarnopolsky MA, Heigenhauser GF, Hill RE, Grant SM. Effects of training duration on substrate turnover and oxidation during exercise. *J Appl Physiol* 1996;81:2182-91.
17. Romijn JA, Klein S, Coyle EF, Sidossis LS, Wolfe RR. Strenuous endurance training increases lipolysis and triglyceride-fatty acid cycling at rest. *J Appl Physiol* 1993;75:108-13.
18. Calles-Escandon J, Goran MI, O'Connell M, Nair KS, Danforth E, Jr. Exercise increases fat oxidation at rest unrelated to changes in energy balance or lipolysis. *Am J Physiol* 1996;270:E1009-14.
19. Blaak EE, Saris WH. Substrate oxidation, obesity and exercise training. *Best Pract Res Clin Endocrinol Metab* 2002;16:667-78.
20. van Aggel-Leijssen DP, Saris WH, Wagenmakers AJ, Senden JM, van Baak MA. Effect of exercise training at different intensities on fat metabolism of obese men. *J Appl Physiol* 2002;92:1300-9.
21. Nicklas BJ, Rogus EM, Goldberg AP. Exercise blunts declines in lipolysis and fat oxidation after dietary-induced weight loss in obese older women. *Am J Physiol* 1997;273:E149-55.

22. Venables MC, Jeukendrup AE. Endurance training and obesity: effect on substrate metabolism and insulin sensitivity. *Med Sci Sports Exerc* 2008;40:495-502.
23. Amati F, Dube JJ, Shay C, Goodpaster BH. Separate and combined effects of exercise training and weight loss on exercise efficiency and substrate oxidation. *J Appl Physiol* 2008;105:825-31.
24. Brandou F, Dumortier M, Garandeau P, Mercier J, Brun JF. Effects of a two-month rehabilitation program on substrate utilization during exercise in obese adolescents. *Diabetes Metab* 2003;29:20-7.
25. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. *Adv Data* 2000:1-27.
26. Ellis KJ, Abrams SA, Wong WW. Monitoring childhood obesity: assessment of the weight/height index. *Am J Epidemiol* 1999;150:939-46.
27. Sunehag AL, Toffolo G, Treuth MS, et al. Effects of dietary macronutrient content on glucose metabolism in children. *J Clin Endocrinol Metab* 2002;87:5168-78.
28. Sunehag AL, Toffolo G, Campioni M, Bier DM, Haymond MW. Effects of dietary macronutrient intake on insulin sensitivity and secretion and glucose and lipid metabolism in healthy, obese adolescents. *J Clin Endocrinol Metab* 2005;90:4496-502.
29. Treuth MS, Sunehag AL, Trautwein LM, Bier DM, Haymond MW, Butte NF. Metabolic adaptation to high-fat and high-carbohydrate diets in children and adolescents. *Am J Clin Nutr* 2003;77:479-89.
30. Otten J, Pitzi Hellwig J, Meyers L. *Dietary (DRI) Reference Intakes: The Essential Guide to Nutrient Requirements*. The National Academies Press: Washington, DC, 2006.
31. Moon JK, Vohra FA, Valerio Jimenez OS, Puyau MR, Butte NF. Closed-loop control of carbon dioxide concentration and pressure improves response of room respiration calorimeters. *J Nutr* 1995;125:220-8.
32. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 1949;109:1-9.
33. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* 1983;55:628-34.
34. Ravussin E, Lillioja S, Anderson TE, Christin L, Bogardus C. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *J Clin Invest* 1986;78:1568-78.
35. Butte NF, Puyau MR, Vohra FA, Adolph AL, Mehta NR, Zakeri I. Body size, body composition, and metabolic profile explain higher energy expenditure in overweight children. *J Nutr* 2007;137:2660-7.
36. Zakeri I, Puyau MR, Adolph AL, Vohra FA, Butte NF. Normalization of energy expenditure data for differences in body mass or composition in children and adolescents. *J Nutr* 2006;136:1371-6.
37. Welle S, Schwartz RG, Statt M. Reduced metabolic rate during beta-adrenergic blockade in humans. *Metabolism* 1991;40:619-22.
38. Kim B. Thyroid hormone as a determinant of energy expenditure and the basal metabolic rate. *Thyroid* 2008;18:141-4.
39. Maffeis C, Armellini F, Tato L, Schutz Y. Fat oxidation and adiposity in prepubertal children: exogenous versus endogenous fat utilization. *J Clin Endocrinol Metab* 1999;84:654-8.
40. Rueda-Maza CM, Maffeis C, Zaffanello M, Schutz Y. Total and exogenous carbohydrate oxidation in obese prepubertal children. *Am J Clin Nutr* 1996;64:844-9.
41. Hawley JA, Lessard SJ. Exercise training-induced improvements in insulin action. *Acta Physiol (Oxf)* 2008;192:127-35.
42. Heled Y, Shapiro Y, Shani Y, et al. Physical exercise enhances hepatic insulin signaling and inhibits phosphoenolpyruvate carboxykinase activity in diabetes-prone Psammomys obesus. *Metabolism* 2004;53:836-41.

Chapter 5

Resistance exercise increases lean body mass and improves hepatic insulin sensitivity in obese adolescents

Gert-Jan van der Heijden

Zhiyue J. Wang

Zili Chu

Gianna Toffolo

Erica Manesso

Pieter J.J. Sauer

Agneta L. Sunehag

Submitted

Abstract

Background: Data are limited on the metabolic effects of resistance exercise (strength training) in adolescents.

Objective: To determine whether a controlled resistance exercise program without dietary intervention or weight loss, reduces body fat accumulation, increases lean body mass, and improves insulin sensitivity and glucose metabolism in sedentary obese Hispanic adolescents.

Participants and Design: Twelve obese adolescents (15.5 ± 0.5y; 35.3 ± 0.8kg/m^2; 40.8 ± 1.5% body fat), completed a 12 wk resistance exercise program (2x1h/wk, exercising all major muscle groups). At baseline and completion of the program, body composition was measured by DXA, abdominal fat distribution by Magnetic Resonance Imaging, hepatic and intramyocellular fat by Magnetic Resonance Spectroscopy, peripheral insulin sensitivity by the Stable Labeled IV Glucose Tolerance Test and hepatic insulin sensitivity by the Hepatic Insulin Sensitivity Index =1000/(GPR*fasting insulin). Glucose production rate (GPR), gluconeogenesis and glycogenolysis were quantified using Stable Isotope-Gas Chromatography/Mass Spectrometry techniques.

Results: All participants were normoglycemic. The exercise program resulted in significant strength gain in both upper and lower body muscle groups. Body weight increased from 97.0 ± 3.8 to 99.6 ± 4.2 kg ($p < 0.01$). The major part (~80%) was accounted for by increased lean body mass (55.7 ± 2.8 to 57.9 ± 3.0 kg; $p \leq 0.01$).Total, visceral, hepatic and intramyocellular fat content remained unchanged. Hepatic insulin sensitivity increased by 24 ± 9% ($p < 0.05$), while peripheral insulin sensitivity did not change significantly. GPR decreased by 8 ± 1% ($p < 0.01$) due to a 12 ±5 % decrease in glycogenolysis ($p < 0.05$).

Conclusion: A controlled resistance exercise program without weight loss increases strength and lean body mass, improves hepatic insulin sensitivity and decreases GPR without affecting total fat mass or visceral, hepatic and intramyocellular fat content.

Introduction

Physical activity is a primary intervention in the combat against obesity and obesity related disorders in children and adolescents. According to the American Academy of Pediatrics, both aerobic (running, biking, swimming) and resistance exercise (strength training) should be part of a multifaceted approach to stimulate exercise and improve fitness in children and adolescents (1).

We and others have demonstrated positive effects of aerobic exercise programs on abdominal fat distribution, hepatic fat content and peripheral as well as hepatic insulin sensitivity in children and adolescents (2-6). In contrast, there are only limited data on the impact of programs containing resistance training alone or in combination with a diet intervention on body composition and insulin sensitivity in children and adolescents (7-11). Increased strength and lean body mass (muscle mass) was observed in three of these studies (7-9), while one (7) reported improved insulin sensitivity. Studies in adults have demonstrated increased strength (12-14), muscle mass (12,13) and insulin sensitivity (12-14) in response to resistance exercise programs.

There is no published information on the impact of a resistance exercise program on hepatic and intramyocellular fat content, or glucose and lipid metabolism in adolescents. Neither has peripheral and hepatic insulin sensitivity (representing different mechanisms to maintain glucose homeostasis) been determined separately in response to resistance training.

Resistance exercise might be an attractive alternative to aerobic training for obese individuals because of its lower aerobic intensity and the positive feedback from the visible strength gain. Studies investigating the effects of resistance exercise programs on metabolic and body composition parameters are crucial in designing strategies that provide various intervention options to prevent obesity related disease.

The aim of the present study was to determine the effect of a controlled resistance exercise program, without additional dietary intervention or weight loss, on body composition, abdominal, hepatic and intramyocellular fat content, peripheral and hepatic insulin sensitivity, and glucose and lipid metabolism in obese adolescents. We focused on sedentary obese Hispanics because of their high risk of obesity related illnesses (15-17).

We hypothesized that in these adolescents, a 12 wk resistance exercise program would increase lean body mass, reduce visceral, hepatic and intramyocellular fat accumulation (IMCL) and improve peripheral and hepatic insulin sensitivity.

Methods and Procedures

Participants

After approval of the protocol by the Baylor College of Medicine Institutional Review Board for Human Subject Research, and the General Clinical Research Center Advisory Board, obese adolescents were recruited by local advertisement. Adolescents were screened and enrolled in the study after written assent from the adolescents and consent from the legal guardian were obtained.

Twelve post pubertal (2 Tanner IV; 10 Tanner V) obese Hispanic adolescents (6m; 6f; 15.5 ± 0.5 y) were studied **(Table 1)**. All participants had BMI > 95th percentile for age (18). Participants had been obese for ≥ 5 years and reported stable body weight for at least 6 months. Only sedentary adolescents were included, i.e. they did not participate in any school or after school organized athletic activities and performed < 45 min light to moderate physical activity/week (by self report).

Table 1. Body composition and fat distribution at baseline and post-exercise (mean ± SE)

	Baseline	Post-Exercise
Weight (kg)	97.0 ± 3.8	99.6 ± 4.2**
BMI (kg/m^2)	35.3 ± 0.7	36.1 ± 0.9**
Body fat %	40.8 ± 1.5	40.2 ± 1.7
Lean body mass (kg)	55.7 ± 2.8	57.9 ± 3.0**
Fat mass (kg)	39.7 ± 1.9	40.3 ± 2.3
Subcutaneous fat (cm^2)	531 ± 24	565 ± 27*
Visceral fat (cm^2)	58 ± 4	55 ± 4
Hepatic fat %	9.2 ± 2.9	9.4 ± 3.1
Intramyocellular fat %$^{\$}$	3.4 ± 0.4	3.4 ± 0.5

Different from baseline: * $p < 0.05$, ** $p < 0.01$.

$^{\$}$Intramyocellular fat % measurements were not obtained in 2 participants

All participants were Hispanic (parents and grandparents of Hispanic descent by self report). They were in good health as determined by a medical history, a physical examination and a standard blood chemistry analysis including blood lipids, liver- and kidney function tests, hemoglobin, hematocrit, hemoglobin A1c and fasting and 2 h post-prandial glucose response. Participants were taking no medications including birth control pills and had no first-degree relatives with diabetes. Adolescents with morbid

obesity (body fat % > 50, sleep apnea, Pickwick syndrome or cor pulmonale) were excluded.

Study Design

Each participant was studied on two occasions: 1) The weekend before start of the exercise program (baseline), 2) Three days after the final exercise session of the 12 wk program (post-exercise). All procedures were identical on both study occasions.

To exclude effects of dietary intake on measurements obtained at baseline versus post-exercise, prior to both studies, each participant received an identical 7 d low-carbohydrate (CHO)/high-fat diet at home (30% CHO, 55% fat, and 15% protein; 20% of the total CHO content as fructose) (19-21). Total energy intake was calculated to correspond to each individual's requirement according to the Institute of Medicine Dietary Reference Intakes (22). We have previously shown that estimating energy requirements based on these criteria results in accurate energy balance (19-21). A pack-out strategy with return and examination of non-consumed food was used (19-21). In order to determine the effect of exercise alone, participants were told not to make lifestyle changes and keep to their habitual diet except adhere to the controlled diet provided the week prior to both study occasions.

On both occasions, the participants were admitted to the General Clinical Research Center at Texas Children's Hospital in the evening before the metabolic study. After dinner and a snack, participants were fasted overnight (except for water) i.e. from 2000 h until completion of the isotope infusion study at 1300 h the next day. Subsequently, the participants were transferred to the radiology department at Texas Children's Hospital for Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) of abdominal, hepatic and intramyocellular fat content.

Resistance Exercise Program

For the duration of 12 wks, participants came to the Physiotherapy Unit at Texas Children's Hospital twice a week for a 1h resistance exercise session. The program was designed according to the guidelines of the American College of Sport Medicine, Sixth Edition (23). Briefly, during wk 1 and 2, the program starts with resistance (weights) corresponding to ~50% of 3 repetitions max (3R max) with 2-3 sets of 8-12 repetitions. The weights and repetitions are then increased gradually according to each individual's ability reaching ~80-85% of 3R max with 3 x 15-20 repetitions during wk 9 to 12.

The 1h session included: 10 min of warm up, 40 min of resistance training and 10 min of cool down. There was at least 1d of rest between sessions. All major muscle groups were trained during each exercise session using one of the alternative exercises for each muscle group outlined in **Table 2**. During the exercise program, first the number of repetitions and subsequently resistance (weight) were increased.

Table 2. Resistance Exercises

Muscle Group	Exercises
Chest	Dumbell (DB) Flys; Push-ups; Chest press
Back	Seated Row; Overhead Press
Triceps	Triceps Extension with Dumbells (DB)
Biceps	Dumbell (DB) Curls; Cable Curls
Shoulders	Lateral Raises; Dumbell (DB) Press
Quadriceps	Leg Extension; Static Lunges; Squats with and without weights
Hamstrings	Dead Lifts with and without weights; Leg Curl with and without weights
Calves	Calf Raises
Gluts	Bridges on Physioball
Abs	Curl ups (straight and obliques)

We present the result of the program as the dynamic strength gain by comparing sets x repetitions and (strength) weights for corresponding exercises at start and completion of the program. Specifically, we evaluated strength improvements in chest, triceps, biceps, quadriceps and hamstring muscles **(Table 3)** Quadriceps strength gain was evaluated using a Biodex isokinetic dynamometer (Shirley, NY) at velocity 180º/ sec. as described by Wiggin et al. (24). All exercise sessions were supervised by trained exercise physiologists. Participants performed no exercise outside the program. Their weight was assessed twice a week in conjunction with the exercise sessions to assure that no weight loss occurred. The purpose of the study was to determine the "chronic" effects of the exercise program and not the "acute" effect of an exercise session. Therefore, the last exercise session took place three days prior to the metabolic and MRI/MRS measurements.

Table 3. Strength improvements in response to the exercise program (mean ± SE).

Muscle Group	Exercise	Pre-Exercise	Pre-Exercise	Post-Exercise	Post-Exercise	% Diff. Pre vs. Post-Exercise	% Diff. Pre vs. Post-Exercise	P-value ↑Weight	P-value ↑ Sets x Reps
		Weight lbs	*Sets x Reps*	*Weight lbs*	*Sets x Reps*	*Weight*	*Sets x Reps*		
Biceps	Cable curls n = 2	12 ± 4	26 ± 2	23 ± 8	54 ± 5	90 ± 11	144 ±39	0.02	0.0009
	Curls w. DB n = 10	n = 12	n = 12	n = 12	n = 12	n = 12	n = 12	n = 12	n = 12
Triceps	Extension w. DB n = 12	12 ± 4	26 ± 2	23 ± 8	49 ± 3	108 ± 26	113 ± 25	0.02	0.0002
		n = 12	n = 12	n = 12	n = 12	n = 12	n = 12	n = 12	n = 12
Chest	Press w. DB n = 7	9 ± 2	30 ± 3	14 ± 3	50 ± 4	84 ± 20	86 ± 21	0.0005	0.0007
	DB Flys n = 4	n = 11	n = 12	n = 11	n = 12	n = 11	n = 12	n = 11	n = 12
	Push ups n = 1								
Hamstrings	Leg Curls or Dead Lifts w.	27 ± 6	31 ± 4	37 ± 8	69 ± 8	33 ± 6	157 ± 53	0.008	0.0003
	weight n = 8	n = 8	n = 12	n = 8	n = 12	n = 8	n = 12	n = 8	n = 12
	Leg Curls or Dead Lifts								
	no weight N = 4								
Quadriceps	Leg ext w. weight n = 2	50; 50	36	70; 70	36	40; 40	0		
	Squats w. weight n = 1	0	16	10	60	(0 -18 lbs)	275		
	Biodex N/m at 180 deg.	RL 88 ± 9	N/A	RL 114 ± 12	N/A	RL 31 ± 7	N/A	RL 0.003	N/A
	n = 9	LL 101 ± 11		LL 115 ± 13		LL 15 ± 6		LL 0.02	
		n = 9		n = 9		n = 9		n = 9	

DB: Dumbells; RL: Right leg; LL: Left leg.

Biodex data were not available in three subjects. Data on leg press and squats represent these three subjects.

Tracers

Deuterium oxide (99% ^{2}H); [$^{2}H_5$]glycerol (99% [^{2}H], 95% [$^{2}H_5$]); [1-^{13}C]glucose (99% [^{13}C]); and [6,6-$^{2}H_2$]glucose (99% [^{2}H], 98% [$^{2}H_2$]) were purchased from Cambridge Isotope Laboratories (Andover, MA). The isotopes were tested for sterility and pyrogenicity by the investigation pharmacy at Texas Children's Hospital (Houston, TX). The infusates were filtered through a Millex GP syringe filter (0.22 µm; Millipore Corporation, Bedford, MN) and stored at 4 ºC for no more than 24–48 h before administration.

Administration of Tracers

On each study occasion, the participants received the following, stable isotopically labeled tracers as previously described (19,20,25).

1. During the overnight fast at 2100, 2300, 0100 and 0300 h, deuterium oxide (a total of 3 g/kg) was administered orally to measure total gluconeogenesis (26).
2. Between 0600 and 1300 h, a simultaneous, primed (60 x the minute infusion rate), constant rate i.v. infusion of [1-^{13}C]glucose (0.33 ± 0 µmol/kg $_{\text{non-bone lean body mass(LBM)}}$ · min) and [$^{2}H_5$]glycerol (0.14 ± 0 µmol/kg $_{\text{LBM}}$ · min) was administered to measure glucose production and the plasma turnover of glycerol, an indicator of lipolysis (19,20,25).
3. The Stable Label Intravenous Glucose Tolerance Test (SLIVGTT) was started at 0900 h after the 0 min blood sample (see below). A bolus injection of glucose, 0.35 ± 0 g/kg $_{\text{LBM}}$ containing 10% [6,6-$^{2}H_2$]glucose, was administered over 90–120 sec to measure insulin sensitivity (19,20,25).

Blood Sampling

Blood samples were obtained just before start of the primed constant rate infusion of the [1-^{13}C]glucose and [$^{2}H_5$]glycerol (designated as t = −180) (13 mL) and subsequently at t = −30, −20, −10, and 0 minutes (8 mL/sample). The injection of the SLIVGTT bolus (after the 0 min sample) was followed by blood sampling (3.6 mL per sample) at +2, 3, 4, 5, 8, 10, 18, 20, 23, 28, 32, 40, 60, 120, 180, and 240 min (19,20,25).

Analyses

Plasma concentrations of glucose, insulin, lipids, leptin, adiponectin and high sensitive C-reactive protein were measured as previously described (5). Non-bone lean body (LBM) and fat mass (FM) were measured by dual-energy x-ray absorptiometry (DXA) (QDR 11.2; Hologic Bedford, MA) (19-21).

Abdominal, hepatic and intramyocellular fat content were measured by Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) using a Philips Achieva 1.5T whole body clinical scanner software release 1.5 (Philips Healthcare, Best, the Netherlands) as previously described in detail (5,19,21). The MR image of abdominal fat i.e. visceral (intra abdominal) and subcutaneous (peripheral) fat content, was acquired in a single transversal slice at the level of the umbilicus (5,19,21). MRI data are expressed as cross-sectional area (cm^2).

A PRESS single voxel technique was used to obtain the liver MR spectra as previously described in detail (5). Data were analyzed using the scanner software and results are expressed as total lipid/water peak area ratio (%). Hepatic fat was considered normal if the MRS lipid peak/water peak was < 5.6 % and high if the MRS lipid peak/water peak was > 5.6 % (27).

A PRESS chemical shift imaging technique was used for measuring IMCL in the soleus muscle (5). Data were analyzed using jMRUI v3.0 (28) with the AMARES algorithm to obtain the peak areas as described by Szczepaniak et al. (29). IMCL is expressed as the relationship between the areas of the IMCL and water peaks, respectively (%).

Calculations

Rates of glucose production and glycerol turnover (an indicator of lipolysis) were calculated under approximate steady-state conditions from the average isotopic enrichments obtained for [$^{13}C_1$]glucose and [$^{2}H_5$]glycerol, respectively, in the samples obtained at -30, -20, -10 and 0 min (19,20,25).

During the same period, the gluconeogenic contribution to glucose (GNG) was determined using $^{2}H_2O$ and the average ^{2}H enrichments of carbons 1,3,4,5,and 6 of glucose (26).

Peripheral insulin sensitivity (the sensitivity of glucose disposition to insulin) was calculated by applying the minimal model to SLIVGTT data (19,20,25,30).

Hepatic insulin sensitivity was calculated in the fasting state by the hepatic insulin sensitivity index (HISI): 1000/[GPR ($\mu mol/kg_{LBM} \cdot min$) x fasting plasma insulin ($\mu U/mL$)], where 1000 is a constant that results in numbers between 1 and 10, as described by Matsuda et al. (31).

Statistical Methods

Power calculations were based on data from our previous study on the effects of an aerobic exercise program (5,6). Thus, 12 subjects would be sufficient to detect changes

of the same magnitude as those found in response to the aerobic exercise program with a power of 0.8 and a type 2 error of 0.05. Data are presented as mean ± SE. Differences between values obtained on the two study occasions were tested by paired t-test. Generalized estimating equations (GEE) (SPSS 17.0) were used to assess the effects of gender and the interaction between the effect of the exercise program and gender. A $p < 0.05$ was considered statistically significant.

Results

Exercise Compliance and Strength Improvement

The participants completed 96 ± 1% of the total 24 exercise sessions. Strength increased for upper body muscles (represented by biceps, triceps and chest) as well as lower body muscles (represented by quadriceps and hamstrings) (**Table 3**).

Energy Intake

Average daily energy intake over the 7-d pre-study diet period were not different at baseline (2911 ± 170 kcal/day) and post-exercise (2853 ± 158 kcal/day). The macronutrient distribution of the intake corresponded to the designed (31 ± 1% CHO, 54 ± 1% fat and 15 ± 1% protein) on both study occasions.

Effect of Resistance Exercise on Body Composition (Table 1)

Total Body Composition (DXA): Total body weight increased ($p < 0.01$). This increase was primarily accounted for (~80%) by an increase in lean body mass (2.1 ± 0.5 kg; $p < 0.01$), while total body fat mass did not change.

Abdominal Fat Distribution (MRI): Visceral fat content did not change significantly, while a minor increase (5 ± 2%), ($p < 0.05$) in subcutaneous fat was observed.

Hepatic Fat Content (MRS) did not change significantly. Seven of twelve adolescents (58%) had high liver fat content (lipid peak/water peak > 5.6 %) (27). In these seven participants, hepatic fat content averaged 13.9 ± 4.3 at baseline and 14.2 ± 4.5% post-exercise (NS). In the five participants with a lipid/water peak < 5.6%, hepatic fat content was 2.7 ± 0.7 at baseline and 2.7 ± 0.9% post exercise (NS).

Intramyocellular Fat Content (MRS) did not change significantly.

Effect of Resistance Exercise on Insulin Sensitivity, Glucose and Lipid Metabolism

Fasting Glucose and Insulin concentrations did not change **(Table 4)**.

Table 4. Biochemical measurements at baseline and post-exercise (mean ± SE)

	Baseline	Post-Exercise
Glucose (mmol/L)	5.1 ± 0.1	5.1 ± 0.1
Insulin (µmol/L)	23.0 ± 1.8	21.0 ± 1.9
Triglycerides (mg/dL)	94 ± 8	94 ± 8
Free Fatty Acids (mmol/L)	0.46 ± 0.02	0.46 ± 0.04
LDL cholesterol (mg/dL)	90 ± 7	95 ± 8
HDL cholesterol (mg/dL)	41 ± 1	42 ± 1
Total cholesterol (mg/dL)	150 ± 8	156 ± 9
Adiponectin (µg/mL)	5.7 ± 0.6	5.4 ± 0.6
Leptin (ng/mL)	46 ± 7	45 ± 6
HS-CRP (mg/L)	1.9 ± 0.4	2.1 ± 0.4

Peripheral Insulin Sensitivity: The average peripheral insulin sensitivity did not change significantly. In 8/12 adolescents, peripheral insulin sensitivity increased while it decreased to the same extent in 4/12 participants (**Figure 1**).

Hepatic Insulin Sensitivity increased by 24 ± 9% ($p < 0.05$) (**Figure 1**).

Glucose Production from Gluconeogenesis and Glycogenolysis: Glucose production rate decreased by 8 ± 1% ($p < 0.01$) accounted for by a 12 ± 5% decrease in glycogenolysis ($p < 0.05$). Gluconeogenesis remained unchanged **(Figure 2)**.

Lipolysis: The exercise program did not have any effect on total glycerol Ra (µmol/min) (Baseline: 225 ± 15 µmol/min; Post: 248 ± 24 µmol/min) or blood lipids **(Table 4)**.

Effect of Resistance Exercise on Adiponectin, Leptin and Hs-CRP

Concentrations of leptin, adiponectin and hs-CRP did not change **(Table 4).**

Effect of Gender

The females had lower weight; height; and lean body mass (all $p < 0.05$) than the males, while their fat% and leptin concentrations were higher (both $p < 0.05$). No other

variable differed between gender at baseline. Except for a greater increase in lean body mass in the males, effects of the exercise program did not differ between gender.

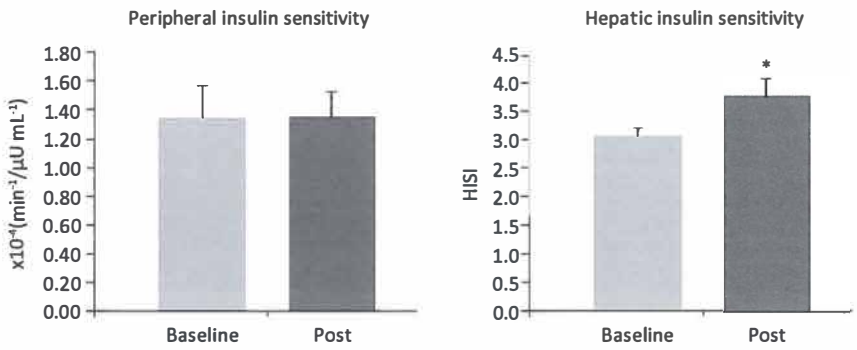

Figure 1. Peripheral Insulin Sensitivity, calculated by the minimal model applied to SLIVGTT data (SI) and Hepatic Insulin Sensitivity, measured by Hepatic Insulin Sensitivity Index (HISI) at baseline and post-exercise (mean ± SE). Different from baseline: * $p < 0.05$, ** $p < 0.01$

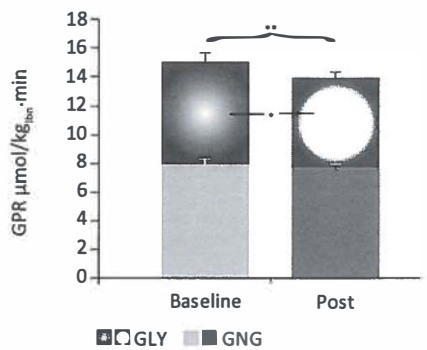

Figure 2. Glucose Production Rate (GPR), consisting of Gluconeogenesis (GNG) (solid part of the bar) and Glycogenolysis (GLY) (upper part of the bar), at baseline and post-exercise (mean ± SE).

Significant differences in GPR are depicted above the bars. Significant difference in GLY is depicted inside the bar. Different from baseline: * $p < 0.05$, ** $p < 0.01$

Discussion

The results of the present study demonstrate that this 12 wk controlled resistance exercise program increased strength, lean body mass and hepatic insulin sensitivity and slightly reduced glucose production in obese Hispanic adolescents. In contrast, the program had no effect on total body, visceral, hepatic and intramyocellular fat or peripheral insulin sensitivity.

Although increased muscle mass and hepatic insulin sensitivity are important results of the resistance exercise program, aerobic exercise seems to have more extensive effects. We demonstrated in a similar group of obese adolescents that the same volume of aerobic exercise significantly reduced total, visceral and hepatic fat content and increased both peripheral and hepatic insulin sensitivity (5,6). These findings are in agreement with other studies in adults and children (3,4,32,33). In contrast, the impact of resistance exercise is less clear (7-13). While most studies reported increased strength and lean body mass (muscle mass) in response to resistance exercise (7-9,12, 13), only a few studies found effects on body fat (7,9,10). Similarly, its influence on insulin sensitivity was inconsistent. Some studies reported improved insulin sensitivity (7,12-14), while others found no metabolic effects of resistance exercise (8,10,11). The methods used in the referenced studies (unlabeled FSIVGTT, unlabeled clamp, oral glucose tolerance test and HOMA-IR) provide a measure of total (i.e. peripheral + hepatic) insulin sensitivity. Using the stable label IVGTT and the Hepatic Insulin Sensitivity Index enabled us to determine peripheral and hepatic insulin sensitivity separately. These measures represent different mechanisms to maintain glucose homeostasis.

Theoretically, one would expect that increased lean body mass (muscle mass) i.e. increased insulin sensitive tissue mass would result in increased insulin sensitivity, primarily peripherally. The increase in lean body mass resulting from our program might have been insufficient to achieve this effect. Neither lean body mass at baseline and post-exercise nor exercise induced change in lean body mass correlated with peripheral or hepatic insulin sensitivity (data not shown). This is in agreement with the referenced studies, where changes in insulin sensitivity (or lack thereof) were independent of changes in lean body mass (7,8,12-14). It has been suggested that resistance training might increase insulin sensitivity as a result of qualitative changes within the muscle (12,13). Brooks et al. (12) and Holten et al. (13) conducted muscle biopsies in adults with type 2 diabetes participating in a whole body (12) or one leg resistance exercise program (13). Brooks et al. (12) demonstrated increased muscle quality (strength/unit

of muscle mass) and increased areas of type I and type II fibers. The increase in type I fiber area correlated significantly with the decrease in insulin resistance. Holten et al. (13) showed increased insulin activity and Glut 4 protein but no effect on markers of oxidative capacity in skeletal muscle. Since muscle biopsies cannot be performed in healthy children and adolescents for ethical reasons, we were not able to pursue potential cellular mechanisms.

A failure to increase lipid oxidation during fasting might lead to intramyocellular fat deposition in obese individuals, subsequently contributing to patterns of insulin resistance (34). Exercise could potentially improve fat oxidation, which might lead to reduced intramyocellular fat content. Indeed, Koopman et. al. (35) demonstrated a decrease in intramyocellular fat directly after a resistance exercise session. However, 120 min after the exercise session the intramyocellular fat content had returned to pre-exercise levels indicating only a short term effect (35). To our knowledge there are no published data on the effects of a resistance exercise program on intramyocellular fat content. We measured intramyocellular fat at baseline and post the exercise program using magnetic resonance spectroscopy but found no changes.

Resistance exercise significantly increased fasting hepatic insulin sensitivity. The mechanism for this effect is not clear. Hepatic fat content measured by magnetic resonance spectroscopy did not change in response to the program. Thus, the improvements in hepatic insulin sensitivity could not be explained by any reduction in hepatic fat accumulation. Heled et al. (36) reported that aerobic exercise training (treadmill) increased hepatic insulin sensitivity due to ameliorated insulin signaling response and inhibited PEPCK activity in the hepatocyte of diabetes prone fat sand rats. Since we could not perform liver biopsies in our healthy adolescents, hepatocellular mechanisms could not be investigated.

Our study population consists of Hispanic adolescents, thus, the studies by Goran's group (7,11,37) in a similar population are of particular interest. In a first study, overweight adolescent Latino boys performed a resistance exercise program, 2 x 1 h/ wk for 16 wks (7). Insulin sensitivity increased by 45%. Lean body mass also increased and body fat % (but not fat mass) decreased. In a later study, overweight adolescent Latino boys and girls were subjected to the same resistance exercise program with an additional diet component aiming at reducing sugar and increasing fiber intake (11). Surprisingly, in this study insulin sensitivity and body composition were completely unaffected. The investigators speculated that the diet intervention might have cancelled out the effect of the exercise. However, a post hoc analysis of the response to the diet

intervention showed no difference between a control group, a diet alone group and the diet + strength training group (37).

A weakness of our study might be that we did not include a non-exercising control group. However, all participants were post-pubertal, therefore, we believe we can exclude any potential effects of growth and maturation on strength gain and muscle mass. For the same reason, pubertal effects on insulin sensitivity are very unlikely. Further, it would be ethically questionable to subject healthy adolescents to comparatively invasive metabolic studies without any intervention.

Resistance exercise resulted in a small decrease (8%) in glucose production due to a decrease (12%) in glycogenolysis while gluconeogenesis remained unchanged. Most likely, the decrease in glucose production has limited clinical relevance in our normoglycemic adolescents with normal glucose tolerance. However, in diabetic adolescents, increased glucose production might result in hyperglycemia (38). Thus, one might speculate that in these individuals, resistance exercise could be a tool to reduce glucose production and subsequently hyperglycemia. The association between decreased glycogenolysis and increased hepatic insulin sensitivity confirms that glycogenolysis is more sensitive to insulin than gluconeogenesis, as suggested by Gastaldelli et al. (39).

Data regarding the effect of resistance exercise on adiponectin, leptin and hs-CRP concentrations are limited (12,40). Brooks et al. (12) found that adiponectin increased and CRP decreased in response to resistance exercise, while Klimcakova et. al. (40) reported unchanged adiponectin and hs-CRP but decreased leptin concentration. We observed no exercise induced changes in these parameters.

In conclusion: Resistance exercise might be an attractive alternative to aerobic exercise for obese adolescents. Increased strength, lean body mass and hepatic insulin sensitivity are important findings. However, the more comprehensive effects of aerobic exercise involving metabolic parameters, body composition and body fat distribution might have a greater potential to prevent obesity related illnesses (5,6). Thus, a program combining resistance and aerobic exercise might be a viable strategy to achieve the positive effects of both types of exercise.

References

1. McCambridge TM, Stricker PR. Strength training by children and adolescents. *Pediatrics* 2008;121:835-40.
2. Nassis GP, Papantakou K, Skenderi K, et al. Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. *Metabolism* 2005;54:1472-9.
3. Gutin B, Owens S. Role of exercise intervention in improving body fat distribution and risk profile in children. *Am J Hum Biol* 1999;11:237-47.
4. Gutin B, Barbeau P, Owens S, et al. Effects of exercise intensity on cardiovascular fitness, total body composition, and visceral adiposity of obese adolescents. *Am J Clin Nutr* 2002;75:818-26.
5. van der Heijden GJ, Wang ZJ, Sauer PJJ, Haymond MW, Rodriguez LM, Sunehag AL. A 12 Week Aerobic Exercise Program Reduces Hepatic Fat Accumulation and Insulin Resistance in Obese, Hispanic Adolescents. *Obesity (Silver Spring) (E-pub ahead of print)* 2009 PMID 19696755.
6. van der Heijden G, Toffolo G, Manesso E, Sauer PJJ, Sunehag AL. Aerobic Exercise Increases Peripheral and Hepatic Insulin Sensitivity in Sedentary Adolescents. *J Clin Endocrinol Metab (E-pub ahead of print)* 2009 PMID 19808855.
7. Shaibi GQ, Cruz ML, Ball GD, et al. Effects of resistance training on insulin sensitivity in overweight Latino adolescent males. *Med Sci Sports Exerc* 2006;38:1208-15.
8. Treuth MS, Hunter GR, Figueroa-Colon R, Goran MI. Effects of strength training on intra-abdominal adipose tissue in obese prepubertal girls. *Med Sci Sports Exerc* 1998;30:1738-43.
9. McGuigan MR, Tatasciore M, Newton RU, Pettigrew S. Eight weeks of resistance training can significantly alter body composition in children who are overweight or obese. *J Strength Cond Res* 2009;23:80-5.
10. Benson AC, Torode ME, Fiatarone Singh MA. The effect of high-intensity progressive resistance training on adiposity in children: a randomized controlled trial. *Int J Obes (Lond)* 2008;32:1016-27.
11. Davis JN, Kelly LA, Lane CJ, et al. Randomized Control Trial to Improve Adiposity and Insulin Resistance in Overweight Latino Adolescents. *Obesity (Silver Spring)* 2009.
12. Brooks N, Layne JE, Gordon PL, Roubenoff R, Nelson ME, Castaneda-Sceppa C. Strength training improves muscle quality and insulin sensitivity in Hispanic older adults with type 2 diabetes. *Int J Med Sci* 2007;4:19-27.
13. Holten MK, Zacho M, Gaster M, Juel C, Wojtaszewski JF, Dela F. Strength training increases insulin-mediated glucose uptake, GLUT4 content, and insulin signaling in skeletal muscle in patients with type 2 diabetes. *Diabetes* 2004;53:294-305.
14. Ishii T, Yamakita T, Sato T, Tanaka S, Fujii S. Resistance training improves insulin sensitivity in NIDDM subjects without altering maximal oxygen uptake. *Diabetes Care* 1998;21:1353-5.
15. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 2006;295:1549-55.
16. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006;118:1388-93.
17. Narayan KM, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. *JAMA* 2003;290:1884-90.
18. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. *Adv Data* 2000:1-27.
19. Sunehag AL, Toffolo G, Campioni M, Bier DM, Haymond MW. Effects of dietary macronutrient intake on insulin sensitivity and secretion and glucose and lipid metabolism in healthy, obese adolescents. *J Clin Endocrinol Metab* 2005;90:4496-502.

20. Sunehag AL, Toffolo G, Treuth MS, et al. Effects of dietary macronutrient content on glucose metabolism in children. *J Clin Endocrinol Metab* 2002;87:5168-78.
21. Treuth MS, Sunehag AL, Trautwein LM, Bier DM, Haymond MW, Butte NF. Metabolic adaptation to high-fat and high-carbohydrate diets in children and adolescents. *Am J Clin Nutr* 2003;77:479-89.
22. Otten J, Pitzi Hellwig J, Meyers L. *Dietary (DRI) Reference Intakes: The Essential Guide to Nutrient Requirements*. The National Academies Press: Washington, DC, 2006.
23. American, College, of, Sports, Medicine. *ACSM's guidelines for exercise testing and prescription*. Philadephia, Penn, 2006.
24. Wiggin M, Wilkinson K, Habetz S, Chorley J, Watson M. Percentile values of isokinetic peak torque in children six through thirteen years old. *Pediatr Phys Ther* 2006;18:3-18.
25. Sunehag AL, Treuth MS, Toffolo G, et al. Glucose production, gluconeogenesis, and insulin sensitivity in children and adolescents: an evaluation of their reproducibility. *Pediatr Res* 2001;50:115-23.
26. Chacko SK, Sunehag AL, Sharma S, Sauer PJ, Haymond MW. Measurement of gluconeogenesis using glucose fragments and mass spectrometry after ingestion of deuterium oxide. *J Appl Physiol* 2008;104:944-51.
27. Szczepaniak LS, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005;288:E462-8.
28. Naressi A, Couturier C, Devos JM, et al. Java-based graphical user interface for the MRUI quantitation package. *MAGMA* 2001;12:141-52.
29. Szczepaniak LS, Babcock EE, Schick F, et al. Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. *Am J Physiol* 1999;276:E977-89.
30. Avogaro A, Bristow JD, Bier DM, Cobelli C, Toffolo G. Stable-label intravenous glucose tolerance test minimal model. *Diabetes* 1989;38:1048-55.
31. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999;22:1462-70.
32. O'Leary VB, Marchett CM, Krishnan RK, Stetzer BP, Gonzalez F, Kirwan JP. Exercise-induced reversal of insulin resistance in obese elderly is associated with reduced visceral fat. *J Appl Physiol* 2006;100:1584-9.
33. Wilmore JH, Despres JP, Stanforth PR, et al. Alterations in body weight and composition consequent to 20 wk of endurance training: The HERITAGE family study. *Am J Clin Nutr* 1999;70:346-52.
34. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. *Diabetes* 2000;49:677-83.
35. Koopman R, Manders RJ, Jonkers RA, Hul GB, Kuipers H, van Loon LJ. Intramyocellular lipid and glycogen content are reduced following resistance exercise in untrained healthy males. *Eur J Appl Physiol* 2006;96:525-34.
36. Heled Y, Shapiro Y, Shani Y, et al. Physical exercise enhances hepatic insulin signaling and inhibits phosphoenolpyruvate carboxykinase activity in diabetes-prone Psammomys obesus. *Metabolism* 2004;53:836-41.
37. Ventura E, Davis J, Byrd-Williams C, et al. Reduction in risk factors for type 2 diabetes mellitus in response to a low-sugar, high-fiber dietary intervention in overweight Latino adolescents. *Arch Pediatr Adolesc Med* 2009;163:320-7.
38. Gastaldelli A, Miyazaki Y, Pettiti M, et al. Separate contribution of diabetes, total fat mass, and fat topography to glucose production, gluconeogenesis, and glycogenolysis. *J Clin Endocrinol Metab* 2004;89:3914-21.
39. Gastaldelli A, Toschi E, Pettiti M, et al. Effect of physiological hyperinsulinemia on gluconeogenesis in nondiabetic subjects and in type 2 diabetic patients. *Diabetes* 2001;50:1807-12.
40. Klimcakova E, Polak J, Moro C, et al. Dynamic strength training improves insulin sensitivity without altering plasma levels and gene expression of adipokines in subcutaneous adipose tissue in obese men. *J Clin Endocrinol Metab* 2

Chapter 6

Body fat percentage and metabolic risk at normal BMI in adolescent girls

Gert-Jan van der Heijden

Zhiyue J. Wang

Zili Chu

Pieter J.J. Sauer

Agneta L. Sunehag

Submitted

Abstract

Background: BMI (kg/m^2) does not provide information about body fat%. Adolescents with BMI < 85th percentile for age are considered lean and at low risk for metabolic complications. Adolescent girls with low BMI can have high body fat (> 27%). Metabolic abnormalities are correlated with body fat content.

Objective: To test the hypothesis that low BMI, high body fat girls are already expressing risk factors of obesity related morbidity.

Participants and Design: Eighteen post-pubertal adolescent girls (13-17y) were studied: 13 had low BMI (<85th percentile/age), high body fat (≥ 27%) **(HiF)** (22.3 ± 0.4 kg/m^2; 32.3 ± 0.7% fat) and 5 had low BMI, low body fat (< 27%) **(LoF)** (19.1 ± 0.7 kg/m^2; 24.4 ± 0.8% fat) (mean±SE). Blood samples were obtained after an overnight fast. Body fat% was measured by DXA and subcutaneous, visceral and hepatic fat by MRI/MRS.

Results: HiF girls had higher visceral ($p < 0.05$) and subcutaneous ($p < 0.01$) fat content compared to LoF girls. Hepatic fat was not elevated in these girls. Fasting insulin, HOMA-IR, leptin and hs-CRP were higher in HiF girls (all $p < 0.05$).

Conclusion: Compared to normal BMI girls with low body fat %, normal BMI girls with body fat > 27% are at increased risk for developing obesity-related co-morbidity. Currently, this group of girls is not detected since only adolescents with BMI > 25 kg/m^2 are considered overweight, thus, at risk of metabolic disorders. Therefore, a validated body fat % measurement needs to be added to the physical screening of adolescent girls.

Introduction

The increasing prevalence of obesity and its co-morbidity in children and adolescents is of great concern. Appropriate screening methods are required to identify children and adolescents at risk of developing obesity and its related diseases.

Metabolic abnormalities are correlated with excess body fat content (1-4), abdominal fat in particular. Of the two components of abdominal fat (visceral and subcutaneous), the visceral (intra abdominal) fat (5-9) seems to play the most important metabolic role.

In children and adolescents, obesity and over weight are most often defined by age related percentiles of Body Mass Index (BMI) [weight in kg/(height in m)^2] (10). BMI might, however, not be an optimal marker of leanness/obesity (11). Adolescents with normal BMI can have high fat mass (11,12). Consequently, using BMI alone for screening, adolescents with normal BMI but high body fat percent (≥ 27%) (13) would inappropriately be classified as lean and thus, at low risk of metabolic disorders.

It is important to determine in adolescents with normal BMI, but high fat %, which body fat depots (i.e. subcutaneous, visceral and hepatic) are enlarged. It is also crucial to determine if these adolescents exhibit other risk factors for obesity-related co-morbidity. If this is the case, the utility of using BMI to detect individuals at risk for metabolic disorder and make decisions about preventive interventions must be reconsidered.

The aims of this study were: To determine whether adolescents with BMI within the normal range but high body fat percentage (HiF) 1) have increased visceral and hepatic fat content compared to adolescents with normal BMI and low body fat percentage (LoF); and 2) exhibit risk factors for obesity-related co-morbidity (insulin resistance, increased concentrations of leptin and hs-CRP and decreased adiponectin concentration).

The study focused on Hispanic adolescent girls because of their relatively high body fat percentage compared to white and black adolescent girls (14) and the high risk for obesity and obesity related illnesses in the Hispanic population (15-17). We studied only adolescent girls because we have encountered the combination of normal BMI and high fat percentage primarily in girls.

Only sedentary participants were included to exclude the metabolic effects of physical activity and fitness on the measurements (3,18).

Subjects and Methods

Subjects

After approval of the protocol by the Baylor College of Medicine Institutional Review Board for Human Subject Research, and the General Clinical Research Center Advisory Board, adolescents were recruited by local advertisement. Adolescents were screened and enrolled in the study after written assent from the participant and consent from the legal guardian were obtained.

Only post-pubertal (Tanner pubertal stage IV-V) sedentary Hispanic adolescent girls were included i.e. they did not participate in any school or after school organized athletic activities and performed < 45 min light to moderate physical activity/week and had parents and grandparents of Hispanic descent by self report. Further, they reported stable body weight for at least 6 months.

In addition, all participants were in good health as determined by a medical history, a physical examination and a standard blood chemistry analysis including blood lipids, liver- and kidney function tests, hemoglobin, hematocrit, hemoglobin A1c and fasting and 2 h post-prandial glucose response. Furthermore, participants were taking no medications including birth control pills and had no first-degree relatives with diabetes. Two groups of adolescent girls participated in the study.

Normal BMI Low Fat (LoF): BMI < 25 kg/m^2 and total body fat < 27%

Normal BMI High Fat (HiF): BMI < 25 kg/m^2 and total body fat ≥ 27%

Eighteen post-pubertal adolescents were studied: 5 LoF and 13 HiF.

BMI cut-off criteria were based on CDC growth charts (10).

Total body fat % criteria were based on data from Shypailo et al. (13), who defined increased total body fat % in girls as > 27%.

Some of the LoF girls participated in a previous study including both males and females (9,19). In these publications no separate data on males and females were presented.

Study design

Participants were admitted to the metabolic research unit at Children's Nutrition Research Center and the General Clinical Research Center at Texas Children's Hospital, Houston, Texas the evening before the study and weight and height measurements were obtained. Dual-energy x-ray absorptiometry (DXA) was obtained at screening and was repeated if more than a month had passed at time of the study.

To exclude effects of dietary intake on measurements, 3 days prior to the study each participant received a controlled diet with a fixed macronutrient distribution (identical in all participant) (20-22). A pack-out strategy with return and examination of non-consumed food was used (20-22). Total energy intake was calculated to correspond to each individual's requirement according to the Institute of Medicine Dietary Reference Intakes (23). After a 12 h overnight fast (except for water), fasting blood samples were obtained.

Subsequently, the participants were transferred to the radiology department at Texas Children's Hospital for Magnetic Resonance Imaging and Spectroscopy (MRI/ MRS) of abdominal and hepatic fat content, respectively.

Body composition analyses

Non-bone lean body, fat mass and body fat percentage were measured at by DXA (QDR 11.2; Hologic Bedford, MA) (20-22).

Abdominal fat content was measured by MRI and intrahepatic fat content by MRS using a Philips Achieva 1.5T whole body clinical scanner, Software Release 1.5, (Best, Holland) (9,20,22) The MR image of abdominal fat i.e. visceral and subcutaneous fat content, was acquired in a single transversal slice at the level of the umbilicus. MRI data are expressed as cross-sectional area (cm^2). Hepatic fat content was expressed as the total lipid/water peak area ratio (%) (24). Hepatic fat was considered normal if the MRS lipid peak/water peak was < 5.6% and high if the MRS lipid peak/water peak was > 5.6% (25).

Biochemical analyses

Plasma concentrations of glucose, insulin, lipids, leptin, adiponectin and high sensitive C-reactive protein (hs-CRP) were measured as previously described (19). Hs-CRP cardiovascular disease risk categories: Hs-CRP < 1.0 mg/L lowest risk; 1.0-3.0 mg/L average risk; > 3.0 mg/L highest risk (26).

Insulin resistance was calculated by the homeostasis model assessment, HOMA-IR (fasting insulin μU/ml x fasting glucose mmol/l /22.5) (27).

Statistical methods

Data are presented as mean ± SE. Differences between groups were assessed by unpaired t-test. Regression analysis was used to test for correlations between variables.

A $p \leq 0.05$ was considered statistically significant. HOMA-IR and hs-CRP data were log transformed.

Results

Clinical characteristics

Demographic characteristics of the participants are presented in **Table 1.**

Table 1. Body composition (mean ± SE)

	LoF	HiF
N	5	13
Age (y)	15.1 ± 0.4	15.0 ± 0.5
Weight (kg)	46.6 ± 2.0	55.5 ± 1.6**
BMI (kg/m2)	19.1 ± 0.7	22.3 ± 0.4**
Body fat %	24.4 ± 0.8	32.3 ± 0.7**
Lean body mass (kg)	33.6 ± 1.7	35.9 ± 0.9
Fat mass (kg)	11.3 ± 0.5	18.2 ± 0.8**

LoF = normal BMI low body fat %, HiF = normal BMI high body fat %;
Different between HiF and LoF participants * $p \leq 0.05$ * $p \leq 0.01$

Body composition and fat distribution

According to the study design, body fat % and fat mass were significantly higher in HiF girls (both $p < 0.01$). Lean body mass did not differ between groups. Although within normal range, BMI was significantly higher in the HiF participants **(Table 1)**. Both visceral and subcutaneous fat content were higher in the HiF compared to the LoF girls ($p < 0.05$ and $p < 0.01$, respectively). Hepatic fat content did not differ between the groups **(Figure 1)**.

Biochemical characteristics

Insulin resistance: All participants were normoglycemic and glucose concentrations were not different between groups. Insulin concentration was significantly higher in HiF compared to LoF girls ($p < 0.05$). As a result, HOMA-IR was significantly higher in HiF girls ($p < 0.05$) **(Figure 2)**.

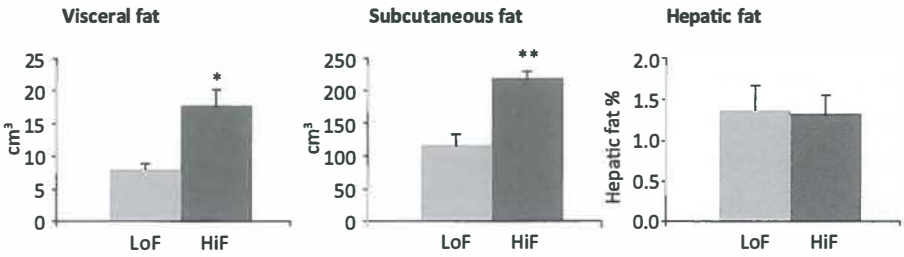

Figure 1. Abdominal fat distribution in normal BMI high fat (HiF), normal BMI low fat (LoF) girls. Different between HiF and LoF participants * $p \leq 0.05$ * $p \leq 0.01$

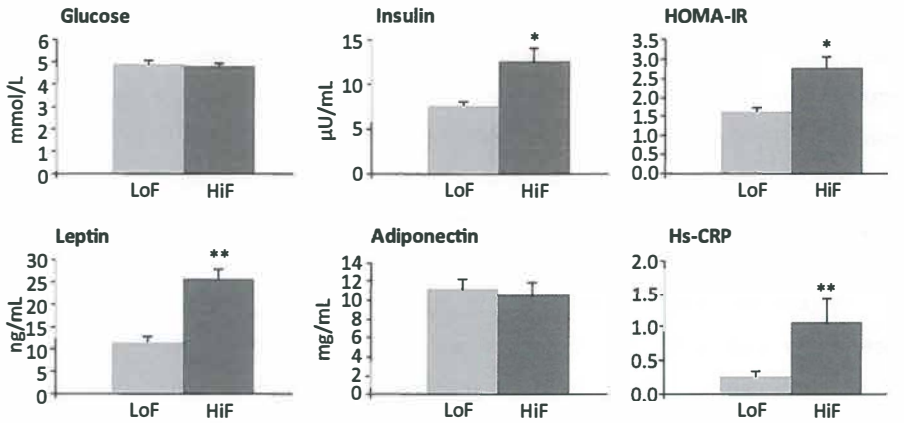

Figure 2. Biochemical parameters in normal BMI high fat (HiF), normal BMI low fat (LoF) girls. Different between HiF and LoF participants * $p \leq 0.05$ * $p \leq 0.01$

Adipokines: Leptin concentration was higher in HiF as compared to LoF girls ($p < 0.01$) **(Figure 2)**.

Adiponectin concentrations were not different in the two groups **(Figure 2)**.

Hs-CRP: Hs-CRP concentration was significantly higher in HiF compared to LoF girls ($p < 0.01$) **(Figure 2)**. No LoF girl had hs-CRP higher than 1 mg/L whereas three HiF girls had hs-CRP between 1 and 3 mg/L

Blood lipids: According to inclusion criteria TG, total cholesterol, HDL, LDL, and FFA were within the normal range in all participants with no difference between the groups.

Correlation Analyses

In LoF girls, HOMA-IR was not correlated with any measured body fat deposits. In contrast, in HiF girls, HOMA-IR was significantly correlated with total body fat % ($R^2 = 0.54$; $p < 0.01$), subcutaneous ($R^2 = 0.47$; $p < 0.05$), visceral ($R^2 = 0.31$; $p = 0.05$) and hepatic fat content ($R^2 = 0.45$; $p < 0.01$).

Discussion

The present study shows that sedentary, post pubertal, adolescent Hispanic girls with normal BMI (< 25 kg/m^2) but high body fat percentage (≥ 27) (HiF) had higher visceral and subcutaneous fat content compared to girls with normal BMI and normal body fat percentage (< 27). All girls had low hepatic fat content with no difference between the groups. Compared to LoF girls, HiF girls exhibited risk factors for obesity-related co-morbidity (higher insulin, leptin and hs-CRP concentrations and HOMA-IR).

It is important to re-emphasize that all participants were sedentary (< 45 min activity per week). Therefore, it is unlikely that the observed differences between groups were due to a difference in activity level.

We and others have reported that in obese adolescents, increased abdominal fat, specifically visceral fat, is an important contributor to obesity related abnormalities (5,7-9,28). However, subcutaneous fat content has also been related to metabolic disorders in pre-pubertal children (28,29). Furthermore, one study found that the combination of high visceral fat and relatively low subcutaneous fat deposits was associated with the most severe metabolic complications in obese adolescents (6). It has been suggested that sufficient fat storage capacity in the subcutaneous depot would prevent accumulation of excess fat in the visceral, hepatic and intramyocellular fat compartments, thus, preventing metabolic disturbances (6). Both subcutaneous and visceral fat was higher in HiF as compared to LoF girls, indicating HiF girls are at a higher risk for developing metabolic disorders.

The high prevalence of fatty liver in obese adolescents (16,30) is of great concern. This severe disease might progress to liver inflammation, fibrosis and cirrhosis (non alcoholic fatty liver disease) (31). We and others have reported that more than 30% of obese adolescents have high liver fat content (9,16,30). However, in the present study, both HiF and LoF girls had normal liver fat content.

While all participants were normoglycemic, fasting insulin concentration and HOMA-IR were higher in HiF girls as compared to LoF girls indicating the HiF girls were insulin resistant. This is a concern since insulin resistance might lead to glucose intolerance and the development of type 2 diabetes. Insulin resistance is also a primary component of the metabolic syndrome (32) and a cardiovascular risk factor (33). Ruderman (34) defined normal height and weight adults with hyperinsulinemia, hypertriglyceridemia and premature coronary heart disease as metabolically obese, normal-weight (MONW). It has recently been shown that MONW adults are at increased risk of developing type 2 diabetes (35). A longitudinal study is needed to determine if HiF girls will turn into MONW adults and indeed develop type 2 diabetes and cardiovascular disease to a greater extend later in life.

In HiF girls, insulin resistance was significantly correlated with both visceral and subcutaneous fat content. Thus, both abdominal fat depots appear to play a role in the deterioration of insulin resistance in this group. In contrast, we and others previously reported that in obese adolescents, insulin resistance was correlated primarily with visceral fat (7-9). This might indicate that the enlargement of the visceral fat compartment associated with increasing fatness has more adverse metabolic consequences than expansion of the subcutaneous fat content (6).

Leptin concentrations were higher in HiF compared to LoF girls. This is in agreement with the reports from Kishimoto et al. (36), who found increased leptin concentrations in Japanese women with normal BMI and high fat % as compared to women with normal BMI and low fat %. These findings might indicate that HiF girls are already leptin resistant (37) and, thus, at increased risk of cardiovascular disease (38). High adiponectin concentrations are purported to have a protective effect on insulin sensitivity partly attributed to its anti inflammatory function (39). Lower adiponectin concentrations in obese as compared have been demonstrated (20,40). In accordance with findings in Japanese women (36), adiponectin concentrations were not different between our LoF and HiF girls. This might suggest that adiponectin is not involved in the increase in insulin resistance in HiF girls.

Concentrations of the inflammatory marker, hs-CRP were higher in HiF as compared to LoF girls. Obese adolescents (19) have been shown to have increased CRP concentrations indicating that low grade chronic whole body inflammation might play a role in obesity related morbidity (41).

In conclusion, sedentary, post pubertal, adolescent Hispanic HiF girls have higher abdominal fat content (subcutaneous and visceral fat) than LoF girls and are exhibiting

increased risk factors for obesity related co-morbidity. Currently, this group of girls is not recognized since only adolescents with BMI > 25 kg/m^2 are considered overweight, thus, at increased metabolic risk. This implies that BMI is not sufficient to detect HiF girls. Therefore a validated body fat % measurement needs to be added to the physical screening of adolescent girls to determine whether preventive interventions are needed.

References

1. Gutin B, Islam S, Manos T, Cucuzzo N, Smith C, Stachura ME. Relation of percentage of body fat and maximal aerobic capacity to risk factors for atherosclerosis and diabetes in black and white seven- to eleven-year-old children. *J Pediatr* 1994;125:847-52.
2. Gutin B, Yin Z, Humphries MC, Hoffman WH, Gower B, Barbeau P. Relations of fatness and fitness to fasting insulin in black and white adolescents. *J Pediatr* 2004;145:737-43.
3. Gutin B, Yin Z, Humphries MC, et al. Relations of body fatness and cardiovascular fitness to lipid profile in black and white adolescents. *Pediatr Res* 2005;58:78-82.
4. Allen DB, Nemeth BA, Clark RR, Peterson SE, Eickhoff J, Carrel AL. Fitness is a stronger predictor of fasting insulin levels than fatness in overweight male middle-school children. *J Pediatr* 2007;150:383-7.
5. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006;444:881-7.
6. Taksali SE, Caprio S, Dziura J, et al. High visceral and low abdominal subcutaneous fat stores in the obese adolescent: a determinant of an adverse metabolic phenotype. *Diabetes* 2008;57:367-71.
7. Caprio S, Hyman LD, Limb C, et al. Central adiposity and its metabolic correlates in obese adolescent girls. *Am J Physiol* 1995;269:E118-26.
8. Liska D, Dufour S, Zern TL, et al. Interethnic differences in muscle, liver and abdominal fat partitioning in obese adolescents. *PLoS ONE* 2007;2:e569.
9. van der Heijden GJ, Wang ZJ, Sauer PJJ, Haymond MW, Rodriguez LM, Sunehag AL. A 12 Week Aerobic Exercise Program Reduces Hepatic Fat Accumulation and Insulin Resistance in Obese, Hispanic Adolescents. *Obesity (Silver Spring) (in press)* 2009.
10. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. *Adv Data* 2000:1-27.
11. Ellis KJ, Abrams SA, Wong WW. Monitoring childhood obesity: assessment of the weight/height index. *Am J Epidemiol* 1999;150:939-46.
12. Freedman DS, Ogden CL, Berenson GS, Horlick M. Body mass index and body fatness in childhood. *Curr Opin Clin Nutr Metab Care* 2005;8:618-23.
13. Shypailo RJ, Butte NF, Ellis KJ. DXA: can it be used as a criterion reference for body fat measurements in children? *Obesity (Silver Spring)* 2008;16:457-62.
14. Ellis KJ, Abrams SA, Wong WW. Body composition of a young, multiethnic female population. *Am J Clin Nutr* 1997;65:724-31.
15. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 2006;295:1549-55.
16. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006;118:1388-93.
17. Narayan KM, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. *JAMA* 2003;290:1884-90.
18. Ischander M, Zaldivar F, Jr., Eliakim A, et al. Physical activity, growth, and inflammatory mediators in BMI-matched female adolescents. *Med Sci Sports Exerc* 2007;39:1131-8.
19. van der Heijden G, Toffolo G, Manesso E, Sauer PJJ, Sunehag AL. Aerobic Exercise Increases Peripheral and Hepatic Insulin Sensitivity in Sedentary Adolescents. *J Clin Endocrinol Metab (in press)* 2009.
20. Sunehag AL, Toffolo G, Campioni M, Bier DM, Haymond MW. Effects of dietary macronutrient intake on insulin sensitivity and secretion and glucose and lipid metabolism in healthy, obese adolescents. *J Clin Endocrinol Metab* 2005;90:4496-502.
21. Sunehag AL, Toffolo G, Treuth MS, et al. Effects of dietary macronutrient content on glucose metabolism in children. *J Clin Endocrinol Metab* 2002;87:5168-78.

22. Treuth MS, Sunehag AL, Trautwein LM, Bier DM, Haymond MW, Butte NF. Metabolic adaptation to high-fat and high-carbohydrate diets in children and adolescents. *Am J Clin Nutr* 2003;77:479-89.
23. Otten J, Pitzi Hellwig J, Meyers L. *Dietary (DRI) Reference Intakes: The Essential Guide to Nutrient Requirements*. The National Academies Press: Washington, DC, 2006.
24. May GL, Wright LC, Holmes KT, et al. Assignment of methylene proton resonances in NMR spectra of embryonic and transformed cells to plasma membrane triglyceride. *J Biol Chem* 1986;261:3048-53.
25. Szczepaniak LS, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005;288:E462-8.
26. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003;107:499-511.
27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-9.
28. Gower BA, Nagy TR, Goran MI. Visceral fat, insulin sensitivity, and lipids in prepubertal children. *Diabetes* 1999;48:1515-21.
29. Maffeis C, Manfredi R, Trombetta M, et al. Insulin sensitivity is correlated with subcutaneous but not visceral body fat in overweight and obese prepubertal children. *J Clin Endocrinol Metab* 2008;93:2122-8.
30. Burgert TS, Taksali SE, Dziura J, et al. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. *J Clin Endocrinol Metab* 2006;91:4287-94.
31. Molleston JP, White F, Teckman J, Fitzgerald JF. Obese children with steatohepatitis can develop cirrhosis in childhood. *Am J Gastroenterol* 2002;97:2460-2.
32. Weiss R, Dziura J, Burgert TS, et al. Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med* 2004;350:2362-74.
33. Ferrannini E, Balkau B, Coppack SW, et al. Insulin Resistance, Insulin Response, and Obesity as Indicators of Metabolic Risk. *J Clin Endocrinol Metab* 2007;92:2885-92.
34. Ruderman N, Chisholm D, Pi-Sunyer X, Schneider S. The metabolically obese, normal-weight individual revisited. *Diabetes* 1998;47:699-713.
35. Meigs JB, Wilson PW, Fox CS, et al. Body mass index, metabolic syndrome, and risk of type 2 diabetes or cardiovascular disease. *J Clin Endocrinol Metab* 2006;91:2906-12.
36. Kishimoto N, Okita K, Takada S, et al. Lipoprotein Metabolism, Insulin Resistance, and Adipocytokine Levels in Japanese Female Adolescents With a Normal Body Mass Index and High Body Fat Mass. *Circ J* 2009.
37. Scarpace PJ, Zhang Y. Leptin resistance: a prediposing factor for diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* 2009;296:R493-500.
38. Wallace AM, McMahon AD, Packard CJ, et al. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). *Circulation* 2001;104:3052-6.
39. Jeffery AN, Murphy MJ, Metcalf BS, et al. Adiponectin in childhood. *Int J Pediatr Obes* 2008;3:130-40.
40. Weiss R, Dufour S, Groszmann A, et al. Low adiponectin levels in adolescent obesity: a marker of increased intramyocellular lipid accumulation. *J Clin Endocrinol Metab* 2003;88:2014-8.
41. de Luca C, Olefsky JM. Inflammation and insulin resistance. *FEBS Lett* 2008;582:97-105.

Chapter 7

Summary, general discussion and future perspectives

&

Samenvatting, algemene discussie en

toekomstperspectief

Summary, general discussion and future perspectives

The rise in obesity related morbidity in children and adolescents requires urgent interventions to prevent and delay obesity and its co-morbidity.

This thesis focused on exercise programs as a tool to counteract the metabolic effects of obesity in the adolescent population. The main objective was to determine in depth, the metabolic effects of a strictly controlled aerobic and resistance exercise program, respectively, (without additional dietary and lifestyle advice and weight loss). The intensity of the programs was designed to accommodate sedentary adolescents.

Each program had a duration of 12 wks. The aerobic exercise program consisted of 48 sessions; 4 x 30 min/wk at ≥ 70 % of VO_{2peak}. The resistance exercise program consisted of 24 sessions; 2 x 1 h/wk; exercising all major muscle groups.

Obesity related morbidity is strongly linked to excess body fat. Particularly specific body fat depots (abdominal, hepatic and intramyocellular fat) are thought to play a metabolically important role. High hepatic fat accumulation (non alcoholic fatty liver) is a highly prevalent disease in obese adolescents that can progress to liver inflammation, fibrosis and cirrhosis. Currently, only limited data are available on the effects of exercise on abdominal, hepatic and intramyocellular fat content.

In **chapter 2**, we report how aerobic exercise affects body composition and body fat distribution (abdominal, hepatic and intramyocellular) and discuss the correlation between insulin resistance and the lipid accumulation in these fat depots in lean and obese adolescents.

Fourteen lean and 15 obese sedentary, post pubertal Hispanic adolescents completed the aerobic exercise program. At baseline and post-exercise, measurements of cardiovascular fitness (treadmill test), whole body composition (Dual energy X-ray Absorptiometry), abdominal fat distribution (Magnetic Resonance Imaging), hepatic and intramyocellular fat content (Magnetic Resonance Spectroscopy) and insulin resistance (HOMA-IR) were obtained.

Compliance with the program was excellent (~ 85 % attendance) resulting in increased fitness in both groups. As intended, body weight did not decrease in lean or obese participants. In obese adolescents, hepatic and visceral fat accumulation decreased while intramyocellular fat content did not change. Insulin resistance decreased, correlating with the decrease in visceral fat ($R^2 = 0.40$). In lean participants, a small increase in body weight accounted for by an increase in lean body mass (muscle mass) was observed.

The finding that an aerobic exercise program reduces hepatic and visceral fat content in obese adolescents independent of weight loss is new and important. It indicates that aerobic exercise has the potential to counteract the development of non alcoholic fatty liver disease even if weight loss is not achieved. The clear interaction between visceral fat and insulin resistance points to an important relationship between this fat depot, in particular, and obesity related metabolic abnormalities.

To our knowledge, no published studies have measured the effect of exercise on peripheral and hepatic insulin sensitivity separately, or reported the impact of training on glucose and lipid metabolism in children or adolescents. Hence, in **chapter 3**, we asked the question: "What is the effect of aerobic exercise on peripheral and hepatic insulin sensitivity and glucose and lipid metabolism?"

To answer this question, peripheral and hepatic insulin sensitivity as well as glucose and lipid kinetics were quantified at baseline and post-exercise using Stable Isotope-Gas Chromatography/Mass Spectrometry techniques in the participants of the aerobic exercise program.

Body weight did not decreased in both groups. As expected, peripheral and hepatic insulin sensitivity were higher in lean compared to obese adolescents. The exercise program increased insulin sensitivity in both groups; peripheral insulin sensitivity by 35 ± 14% in the lean and 59 ± 19% in the obese participants and hepatic insulin sensitivity by 19 ± 7% in the lean and 23 ± 4% in the obese adolescents. Glucose production, gluconeogenesis and glycogenolysis were not different between groups. In response to the exercise program glucose production decreased by 3 ± 1% and 4 ± 1% in lean and obese participants, respectively. Gluconeogenesis remained unchanged, while glycogenolysis decreased slightly but significantly in the obese group.

Collectively, our results indicate that a moderate aerobic exercise program without weight loss substantially improved both peripheral and hepatic insulin sensitivity. Thus, this program could be a useful tool to prevent the development of obesity related illness in Hispanic adolescents. The small decrease in glucose production is probably of limited clinical relevance in our normoglycemic, glucose tolerant participants. However, it is worth exploring if an exercise-induced decrease in glucose production can prevent or decrease hyperglycemic episodes in diabetic individuals.

In **chapter 4**, we discuss whether the metabolic changes resulting from the aerobic exercise program described in chapter 2 and 3 were related to increased 24 h energy expenditure and fat oxidation.

Thirteen lean and 15 obese sedentary, post pubertal, Hispanic adolescents participating in the aerobic exercise program underwent 24 h room-calorimetry before start of the program and at least 24 h after their final exercise session. We found that aerobic exercise without weight loss did not affect total, basal, sleep and awake sedentary energy expenditure in either obese or lean adolescents. In lean participants fat oxidation increased, while in the obese, oxidation rates were not affected.

Thus, in obese adolescents, changes in whole body energy expenditure and substrate oxidation rates are not required to reduce visceral and hepatic fat content and increase peripheral and hepatic insulin sensitivity

The previous chapters focused on the positive effects of 12 wks moderate aerobic exercise. Resistance exercise might be an attractive alternative to aerobic training for obese individuals because of its lower aerobic intensity and the positive feedback from the visible strength gain. In **chapter 5**, we present the results of the 12 wk resistance exercise (strength training) program on whole body composition, fat distribution, peripheral and hepatic insulin sensitivity and glucose and lipid metabolism in obese adolescents. Furthermore, the effects of resistance exercise are compared with those of aerobic exercise. We asked the question:"Is there an optimal exercise intervention for obese adolescents?"

To this end, twelve obese, sedentary, post pubertal, Hispanic adolescents were enrolled in the resistance exercise program. At baseline and post-exercise, identical measurements were obtained as described in chapter 2 and 3.

The program resulted in increased strength, muscle mass and hepatic (but not peripheral) insulin sensitivity and decreased glucose production. A small increase in subcutaneous fat was also observed, while total, visceral, hepatic and intramyocellular fat content did not change.

Increased muscle mass and hepatic insulin sensitivity and decreased glucose production are important positive outcomes of the resistance exercise program. However, aerobic exercise seemed to have more comprehensive effects involving increased peripheral insulin sensitivity, and decreased total body, visceral and hepatic fat accumulation (chapter 2 and 3). Therefore, our results suggest that aerobic exercise might be the preferable exercise for obese adolescents.

In the studies described in chapters 2, 3, 4 and 5, lean adolescents included in the study were selected based on BMI (< 25 kg/m^2) and body fat percentage (< 27). The reason for the additional body fat percentage (%) criteria was that we wanted to compare strictly lean with strictly obese adolescents and BMI is not an optimal marker

of leanness/obesity. During screening procedures we noticed that a large percentage of Hispanic adolescent girls were lean according to BMI criteria but had a relative high body fat %. Because obesity related metabolic abnormalities are correlated with excess body fat, this led to the question that is addressed in **chapter 6**: "Are adolescent girls with normal BMI but high body fat % at risk for obesity related morbidity?"

Whole body composition (DXA), abdominal fat distribution (MRI), hepatic fat content (MRS), insulin resistance (HOMA-IR), adipokines, hs-CRP and blood lipids were measured in 2 groups of sedentary post-pubertal Hispanic adolescent girls: Five lean low fat (LoF) (BMI < 25 kg/m^2 and body fat % < 27) and thirteen lean high fat (HiF) (BMI < 25 kg/m^2 and body fat % ³ 27). Compared to LoF girls, HiF girls had higher visceral and subcutaneous fat content, while hepatic fat was low in both groups. In addition, HiF girls exhibited risk factors for obesity related morbidity (higher insulin, leptin, and hs-CRP concentrations and insulin resistance).

We conclude that compared to Hispanic girls with a normal BMI and low body fat %, girls with normal BMI and a high body fat % exhibit several metabolic risk factors. Currently this group of girls is not recognized since only adolescents with BMI > 25 kg/m^2 are considered overweight, thus, at increased metabolic risk. Therefore a validated body fat % measurement needs to be added to the physical screening of adolescent girls to determine whether preventive interventions are needed.

Collectively, the data from our studies demonstrate that controlled aerobic and resistance exercise programs, without dietary intervention or weight loss, counteract a number of obesity related metabolic abnormalities. Therefore, both types of exercise are important tools to prevent and delay obesity related morbidity in the adolescent population. The intensity of the exercise programs was moderate and lean and obese adolescents, who were all used to a sedentary life style, were able to comply very well. Thus, these programs could be included in the day to day life of adolescents.

A number of studies have demonstrated positive effects of aerobic and resistance exercise in various groups of healthy and sick adults. Investigations on the impact of exercise have also been published in children and adolescents but comprehensive data on metabolism and body fat distribution are limited. Although exercise has been shown to be beneficial in all age groups, it is of utmost importance to stimulate an interest for physical activity and exercise in childhood. Metabolic abnormalities of obesity are already present in adolescence and early prevention might counteract the development of obesity and obesity related abnormalities in this age group. Furthermore it might

curb the development of overweight in adolescence to adult overweight and its related cardiovascular risk factors

Although both aerobic and resistance exercise programs resulted in positive metabolic effects, obese adolescents remained more insulin resistant as compared to their lean counterparts at the end of the program. It would have been surprising if 12 wks aerobic or resistance training completely normalized the metabolic profile in obese adolescents. However, longitudinal studies are needed to determine if exercise alone is able to achieve this goal. In addition, resistance and aerobic exercise both result in positive, but partly different metabolic changes. Consequently, a program combining resistance and aerobic exercise might be the optimal strategy to achieve the benefits of both types of exercise. This approach would also provide more variation and stimulation, thus increasing the likelihood of long term success. Furthermore, it is possible that exercise alone is not sufficient to completely reverse the deteriorated metabolic profile of an obese adolescent. Combining exercise with an easy to comply with dietary intervention leading to sustained weight loss might further improve the metabolism in obese adolescents.

Several specific findings with potential clinical implications described in this thesis warrant further investigation:

- The promising potential of aerobic exercise alone (without weight loss or dietary intervention) to prevent and treat non alcoholic fatty liver disease in obese adolescents should be tested in a population with biopsy proven non alcoholic fatty liver disease.
- Exercise led to reduced glucose production in our, glucose tolerant, participants. Studies in adolescents with glucose intolerance and type 2 diabetes are needed to determine whether the exercise induced decrease in glucose production is reproduced and leads to a subsequent reduction in blood glucose concentration.
- The mechanisms behind the interplay between specific body fat depots (visceral, subcutaneous, hepatic and intramyocellular) and metabolic parameters such as peripheral and hepatic insulin sensitivity, gluconeogenesis, and glycogenolysis could not be fully elucidated. Processes on the cellular level might be involved. However, these avenues could not be pursued since muscle, liver and abdominal fat biopsies can not be performed in healthy adolescents for ethical reasons. Studies using isotopic labeling in combination with advanced magnetic resonance imaging might provide new information.

We present in depth the positive metabolic effect of exercise in lean and obese adolescents. In order to actually prevent or delay the development of obesity related illnesses, activity programs need to be implemented on a population wide scale. Further, incorporation of physical activity in daily life routines has to be facilitated and encouraged. Active involvement of parents and educators is crucial to promote physical activity in children and adolescents. On a governmental level, legislation has to be passed and programs started to create an environment that directs society to be more active.

Samenvatting, algemene discussie en toekomstperspectief

De toename van obesitas in kinderen en adolescenten maakt interventies ter preventie van obesitas en de hieraan gerelateerde ziektebeelden noodzakelijk. De doelstelling van dit proefschrift was om in detail de metabole effecten vast te leggen van een strikt gecontroleerd aeroob (o.a. hardlopen en fietsen) of kracht trainingsprogramma (o.a. gewichtheffen) zonder aanvullend dieetadvies en gewichtsverlies.

Zowel het aeroob als het kracht trainingsprogramma waren ontwikkeld met een intensiteit die uitvoerbaar was voor inactieve, sedentaire adolescenten. Beide trainingsprogramma's hadden een duur van 12 weken. Het aerobe programma bestond uit 48 sessies; 4 x 30 minuten/week ≤ 70 % van VO_{2peak}. Het kracht trainingsprogramma bestond uit 24 sessies; 2 x 1 uur/week, waarin de voornaamste spiergroepen werden getraind.

Aan obesitas gerelateerde morbiditeit is sterk gecorreleerd met excessief lichaamsvet. In het bijzonder wordt een belangrijke metabole rol toegedicht aan enkele specifieke vetdepots (abdominaal, lever en intramyocellulair). Een grote stapeling van levervet (niet-alcoholische leververvetting) is een zeer prevalent ziektebeeld in obese adolescenten dat kan leiden tot leverinflammatie, fibrose en cirrose. Op dit moment is er slechts in beperkte mate informatie beschikbaar omtrent de effecten van lichamelijke training op abdominaal, lever en intramyocellulair vet.

In **hoofdstuk 2** wordt beschreven hoe aerobe training de lichaamssamenstelling en vetdistributie beïnvloedt in adolescenten met normaal gewicht of obesitas. Bovendien wordt de relatie tussen insulineresistentie en specifieke vetdistributie besproken.

Veertien adolescenten met normaal gewicht en 15 adolescenten met obesitas werden geïncludeerd in het aerobe trainingsprogramma. Alle participanten waren sedentair, post-pubertaal en Latijns Amerikaans (Hispanic). Voor aanvang en na afloop van het programma werden metingen verricht ter analyse van cardiovasculaire fitness (inspanningstest), algehele lichaamssamenstelling (Dual Energy X-ray Absorptiometry) abdominale vetdistributie (Magnetic Resonance Imaging), hepatisch en intramyocellulair vet (Magnetic Resonance Spectroscopy) en insuline resistentie (HOMA-IR).

Het programma werd uitstekend gevolgd (85% aanwezigheid), resulterend in toegenomen fitness in beide groepen. Zoals beoogd was er geen gewichtsafname. In de obese adolescenten nam hepatisch en visceraal vet af terwijl intramyocellulair vet niet veranderde. De afname van visceraal vet was gecorreleerd met de afname van

insulineresistentie ($R^2 = 0.40$). In de participanten met normaal gewicht werd een kleine toename in vetvrij lichaamsgewicht (spiermassa) geobserveerd.

De bevinding dat aerobe training resulteerde in een afname van lever en visceraal vet onafhankelijk van gewichtsverlies impliceert dat aerobe training zonder gewichtsverlies mogelijk in staat is de ontwikkeling van niet-alcoholische leververvetting tegen te gaan. De duidelijke interactie tussen visceraal vet en insulineresistentie wijst op een belangrijk samenspel tussen dit vetdepot in het bijzonder en de aan obesitas gerelateerde metabole afwijkingen.

Voor zover het ons bekend is, is er geen eerder gepubliceerde studie die het effect van training op afzonderlijk, perifere en hepatische insuline sensitiviteit heeft gemeten. Bovendien is er niet eerder gerapporteerd over de invloed van training op glucose en vet metabolisme in kinderen en adolescenten.

In **hoofdstuk 3** beantwoorden wij de vraag: "Wat is het effect van aerobe training op perifere en hepatische insuline sensitiviteit en glucose en vet metabolisme."

Voor aanvang en na afloop van het trainingsprogramma werd perifere en hepatische insulinesensitiviteit en glucose en vet kinetiek gekwantificeerd met gebruik van stabiele isotopen gaschromatografie/ massaspectrometrie technieken. Metingen werden verricht in de participanten die deelnamen aan het aerobe trainingsprogramma.

Lichaamsgewicht bleef onveranderd in beide groepen. Zoals verwacht was perifere en hepatische insulinesensitiviteit hoger in adolescenten met normaal gewicht in vergelijking tot de kinderen met obesitas. Het trainingsprogramma resulteerde in een toename van insulinesensitiviteit in beide groepen; perifere insulinesensitiviteit nam toe met 35 ± 14% in de participanten met normaal gewicht en 59 ± 19% in de obese adolescenten, hepatische insuline sensitiviteit nam toe met 19 ± 7% in de adolescenten met normaal gewicht en met 23 ± 4% in de obese participanten. Glucoseproductie, gluconeogenese and glycogenolyse verschilden niet tussen beide groepen. Als gevolg van het trainingsprogramma nam glucoseproductie af met 3 ± 1% en 4 ± 1% in respectievelijk participanten met normaal gewicht en adolescenten met obesitas. Gluconeogenese bleef onveranderd terwijl een geringe maar significante afname in glycogenolyse werd geobserveerd in obese adolescenten.

Collectief tonen de resultaten aan dat een aeroob trainingsprogramma met matige intensiteit en zonder gewichtsverlies leidt tot een substantiële toename in perifere en hepatische insulinesensitiviteit. Derhalve, kan dit programma een waardevol hulpmiddel zijn ter preventie van de ontwikkeling van aan obesitas gerelateerde ziekten, zoals type 2 diabetes.

De bescheiden afname in glucoseproductie is waarschijnlijk van beperkte klinische relevantie. Echter, het is de moeite waard te exploreren of een training geïnduceerde afname in glucoseproductie hyperglycemische episoden kan voorkomen in glucose-intolerante personen.

In **hoofdstuk 4** bestudeerden wij of de aerobe training geïnduceerde metabole veranderingen beschreven in hoofdstuk 3 en 4 waren gerelateerd aan een toegenomen 24 uurs energieverbruik en vetoxidatie.

Voor aanvang en na afloop van het aerobe trainingsprogramma ondergingen 13 participanten met normaal gewicht en 15 met obesitas 24 uurs calorimetrie in een speciaal voor deze meting ontworpen kamer (room calorimetry).

Wij constateerden dat aerobe training zonder gewichtsverlies geen effect had op totaal, basaal, slaap en sedentair energieverbruik in zowel de adolescenten met normaal gewicht als obesitas. In participanten met normaal gewicht nam vetoxidatie toe, terwijl substraatoxidatie in de adolescenten met obesitas niet veranderde.

Dus, in adolescenten met obesitas zijn chronische training geïnduceerde veranderingen in energieverbruik en substraatoxidatie niet noodzakelijk om een reductie van visceraal en hepatisch vet en een toename van perifere en hepatische insulinesensitiviteit te bewerkstelligen.

De voorgaande hoofdstukken concentreerden op de positieve effecten van 12 weken aerobe training. Krachttraining is mogelijk een aantrekkelijk alternatief op aerobe training voor obese adolescenten vanwege de lagere aerobe intensiteit en de positieve feedback van zichtbare krachttoename.

In **hoofdstuk 5** bediscussiëren wij de resultaten van 12 weken krachttraining op lichaamssamenstelling, vetdistributie, insulinesensitiviteit en glucose en vet metabolisme. Bovendien worden de effecten van krachttraining vergeleken met het resultaat van aerobe training. Wij stelden ons de vraag: "Is er een optimale trainingsinterventie voor obese adolescenten."

Twaalf obese sedentaire post-pubertale Latijns Amerikaanse adolescenten participeerden in het trainingsprogramma. Voor aanvang en na afloop van het programma werden identieke metingen verricht zoals beschreven voor de aerobe interventie (hoofdstuk 2 en 3).

De training resulteerde in toegenomen spierkracht, spiermassa, hepatische, maar geen perifere, insulinesensitiviteit en een afname in glucoseproductie. Terwijl totaal, visceraal, hepatisch en intramyocellulair vet niet veranderden was er een minimale toename in subcutaan vet.

De toegenomen kracht, spiermassa en hepatische insulinesensitiviteit en afgenomen glucoseproductie zijn belangrijke positieve resultaten. Echter, aerobe training lijkt een veelomvattender effect te hebben met een additionele toename in perifere insulinesensitiviteit en een afname in totaal, visceraal en hepatisch vet (hoofdstuk 2 en 3). Derhalve, suggereren onze resultaten dat aerobe training de voorkeur verdient voor obese adolescenten.

In de studies beschreven in hoofdstuk 2, 3, 4 en 5 zijn de adolescenten met normaal gewicht geselecteerd op basis van Body Mass Index (< 25 kg/m²) en lichaamsvetpercentage (< 27) criteria. De beweegreden voor de toevoeging van het lichaamsvetpercentage (vet %) criterium is dat BMI alleen, geen optimale marker is voor lichaamssamenstelling. In deze studies wilden wij adolescenten met een nomaal gewicht en vet % vergelijken met adolescenten met een obees gewicht en vet %. Gedurende de screeningprocedures viel op dat een groot percentage Latijns Amerikaanse adolescente meisjes een normaal gewicht had op basis van BMI, maar tegelijkertijd een relatief hoog vet %. Aangezien aan obesitas gerelateerde metabole afwijkingen correleren met excessief lichaamsvet leidde dit tot de vraag die wordt beantwoord in **hoofdstuk 6**: "Lopen adolescente meisjes met een normale BMI maar een hoog vet % gevaar voor aan obesitas gerelateerde morbiditeit?"

Algehele lichaamssamenstelling (DXA) abdominale vetdistributie (MRI), hepatisch vet (MRS), insulineresistentie (HOMA-IR), adipokines, hs-CRP and lipiden werden gemeten in 2 groepen sedentaire post-pubertale Latijns Amerikaanse adolescente meisjes: Vijf meisjes met normaal gewicht en normaal vet % (lean low fat (LoF), BMI < 25 kg/m² en vet % < 27) en 13 met normaal gewicht maar hoog vet % (lean high fat (HiF), BMI < 25 kg/m² en vet % ≤ 27).

In vergelijking tot LoF meisjes, hadden HiF meisjes hoger visceraal en subcutaan vet, terwijl hepatisch vet laag was in beide groepen. Hiernaast etaleerden HiF meisjes risico factoren voor aan overgewicht gerelateerde morbiditeit (hogere insuline, leptine, en hs-CRP concentraties en insulineresistentie.

Wij concluderen dat meisjes met een normale BMI en hoog vet % een aantal metabole risico factoren ten toon spreiden in vergelijking tot meisjes met een normaal BMI en vet %. Op dit moment wordt deze groep meisjes niet onderkend aangezien alleen adolescenten met een BMI > 25 kg/m² worden ingedeeld in de categorie overgewicht en dus als risicogroep voor aan overgewicht gerelateerde morbiditeit. Derhalve opperen wij dat een gevalideerde vet % meting moet worden toegevoegd

aan de lichamelijke screening van Latijns Amerikaanse adolescente meisjes, om vast te stellen of preventieve interventies moeten worden gestart.

Gezamenlijk tonen onze studies aan dat een gecontroleerd aeroob of kracht trainingsprogramma zonder dieetinterventie of gewichtsverlies een significant aantal aan obesitas gerelateerde metabole afwijkingen tegengaat. Zodoende zijn beide soorten training belangrijke hulpmiddelen ter preventie en vertraging van de ontwikkeling van de co-morbiditeit van obesitas in de adolescentie. De intensiteit van de trainingsprogramma's was gematigd en sedentaire adolescenten met en zonder obesitas volgden de programma's uitstekend. Dit duidt erop dat deze programma's kunnen worden geïmplementeerd in het dagelijks leven van adolescenten.

Een aantal studies heeft aangetoond dat aerobe training en krachttraining positieve effecten hebben in verschillende groepen gezonde en zieke volwassenen. Studies inzake het effect van training bij kinderen zijn ook gepubliceerd, maar uitvoerige data betreffende de stofwisseling en lichaamsvetdistributie zijn schaars. Lichamelijke training is heilzaam in alle leeftijdscategorieën. Echter, het is van groot belang om interesse in lichamelijke activiteit en training te stimuleren op de kinderleeftijd. Metabole afwijkingen zijn reeds aanwezig in de adolescentie en vroege preventie kan de ontwikkeling van obesitas en de hieraan gerelateerde morbiditeit tegengaan in deze leeftijdscategorie. Bovendien kan het mogelijk de ontwikkeling van overgewicht gedurende adolescentie naar volwassen overgewicht en de hieraan gerelateerde cardiovasculaire risicofactoren tegengaan.

Ondanks het feit dat beide programma's resulteerden in positieve metabole effecten, bleven obese adolescenten meer insulineresistent in vergelijking tot adolescenten met normaal gewicht. Het zou verrassend zijn geweest indien 12 weken aerobe of kracht training geresulteerd had in een volledige normalisatie van het metabole profiel in obese participanten. Echter, longitudinale studies zijn nodig om vast te stellen of louter training in staat is dit doel te bereiken. Hiernaast is een programma dat aerobe training en krachttraining combineert een potentiële strategie om de positieve effecten van beide soorten training te behalen. Deze aanpak zou ook zorgen voor meer variatie en stimulatie wat mogelijk de kans op lange termijn opvolging van het programma vergroot. Uiteindelijk is het mogelijk dat training alleen onvoldoende is om het verslechterde metabole profiel van een obese adolescent volkomen teniet te doen. Training gecombineerd met een gemakkelijk op te volgen dieetinterventie leidend tot chronisch gewichtsverlies zou kunnen resulteren in een verder toenemende metabole verbetering in obese adolescenten.

Een aantal specifieke bevindingen met potentiële klinische implicaties verdient nader onderzoek.

- De mogelijkheid dat aerobe training (zonder gewichtsverlies of dieetinterventie) niet-alcoholische leververvetting tegen kan gaan in obese adolescenten is veelbelovend en moet bestudeerd worden in een populatie met door middel van biopsie bewezen leververvetting.
- Training leidde tot een afname in glucoseproductie in onze glucosetolerante participanten. Studies in adolescenten met glucoseintolerantie en type 2 diabetes zijn nodig om te verifiëren of de door training geïnduceerde afname in glucoseproductie leidt tot een afname van serumglucose waarden.
- De mechanismen achter de interactie tussen specifieke lichaamsvetdepots (visceraal, subcutaan en intramyocellulair) en metabole parameters zoals perifere en hepatische insulinesensitiviteit, gluconeogenese en glycogenolyse konden niet volledig worden verklaard. Processen op cellulair niveau spelen hierbij waarschijnlijk een rol. Echter, deze wegen konden niet worden geëxploreerd aangezien spier-, lever- en abdominaalvetbiopten vanwege ethische redenen niet mogen worden uitgevoerd in gezonde adolescenten. Studies met behulp van stabiele isotopen en geavanceerde MRI technieken zullen mogelijk nieuwe informatie verschaffen.

Wij presenteren in detail de positieve metabole effecten van lichamelijke training in adolescenten met normaal gewicht en obesitas. Om de ontwikkeling van de aan obesitas gerelateerde ziekten substantieel tegen te gaan en te voorkomen moeten activiteitenprogramma's worden geïmplementeerd op een bevolkingswijde schaal. Bovendien moet de verwerking van lichamelijke activiteit in de dagelijkse routine worden gefaciliteerd en aangemoedigd. De actieve betrokkenheid van ouders/ opvoeders en docenten is cruciaal ter promotie van lichamelijke activiteit in kinderen en adolescenten. Echter, op regeringsniveau moeten wetsvoorstellen worden ingediend en programma's worden opgestart ter verwezenlijking van een stimulerende leefomgeving die leiden tot een actievere bevolking.

Dankwoord

Finally, the perfect opportunity to thank everybody that worked so hard on the studies that resulted in this thesis. Thank you all very much for your help, efforts and all the good times we shared over the past years!

In particular, I want to thank the most important people of the project, the boys and girls that took part in these intensive studies. Without your time and commitment this project could simply not have taken place. Thank you!

Prof. dr. P.J.J. Sauer, beste Pieter, ik had mij geen betere en enthousiastere promotor kunnen wensen. Vanaf het het bericht dat het Children's Nutrition Research Center een geweldige plek is om te werken, tot en met de non-stop dagen in Groningen en Houston heb ik voortdurend de beste begeleiding gekregen. Bedankt voor de kritische blik en de vastberadenheid. Hiernaast, nogmaals dank voor alle flexibiliteit die ik het afgelopen jaar heb gekregen.

Prof. dr. A.L. Sunehag, dear Agneta, the moment we met I knew this thesis would be fine. Your enthusiasm and drive to carefully explain everything about stable isotopes was transferred immediately and made me study books I never thought I would consider interesting. The opportunities you gave me in all aspects of research are numerous and I am very grateful for this experience. I hope to keep our friendship, collaboration and Margaritas at Pappasito's in the coming years.

Prof. dr. H.A. Delemarre–van de Waal, Prof. dr. R.P. Stolk and Prof. dr. D.M. Bier, I am very thankful for your time and effort to review this thesis and your participation in de leescommissie.

Prof dr. M.W. Haymond, dear Morey, I want to extend my gratitude for the advice you gave me during my stay in Houston. Moreover, the fact that you managed to stay such an upbeat person during your very busy years makes you a great example for me.

Prof dr. D.M. Bier, I am obliged to you for allowing me to work and experience the diversity in research at the Children's Nutrition Research Center.

Prof dr. W.C. Heird, I am thankful that you granted me a position in the Postdoctoral Research Training Program at the CNRC. Furthermore, thank you very much for your hospitality, in particular the thanksgiving dinners.

A big part of life in Houston took place at work and this is the place were I got to meet great new friends and colleagues. Help and advice at work, good discussions, exploring downtown, coffee at Starbucks, Fridays at the Gingerman and the rodeo BBQ cook -off, are all great memories. During trips to Houston over the past year you allowed me to stay at your house and with your family, and I will be happy to return this favour. Mahmoud and family, Trinna, Jean, Shaji and family, Moniek and Robert, Sascha and Patrycja, Murali and family, Jason, Luisa and Karen, I am lucky to have met all of you. It was great!

The success of the 12 week exercise programs is the result of many enthusiastic people working closely together.
Exercise physiologists Mitzi, Cynthia, Ashley, Veronica and Jennifer; research nurses, Amy, Cindy, Linda, Shawn, Jenyce and other nurses at the GCRC and MRU; research coordinator Janette Gonzalez; dietician Ann McMeans; the staff of the amazing calorimeter core laboratory, Prof. dr. N. Butte, Maurice Puyau, Ann Adolph and Firoz Vohra. A sincere thank you.
Moreover, Susan, Marcia, Shaji and Dan, thank you for providing your technical assistance and sharing your knowledge with me, and Prof. dr. O'Brian Smith, my gratitude for advice on statistical analysis.

Collaboration was key for the realisation of this project. The MRI acquisition and analysis was performed by Dr. Z.J. Wang and Dr. Z. Chu. Dear Jerry and David, thank you for the busy and good Sundays.
Prof. dr. G.M. Toffolo and Drs E. Manesso, I want to extend my gratitude for the ongoing collaboration in the assessment of insulin sensitivity and I hope to visit you in Italy one day.

Karen Jones and Han Marra I am obliged to you for being so helpful over the years.

Tot slot vrienden en familie.

Steven, Gijs, Peter, Auke, Oscar en Thomas, Tim en Olaf, en de hele geneeskunde groep, hartstikke bedankt voor de mail, telefoontjes en de mooie dagen en vakanties in Texas en de rest van de VS.

Gabin en Oscar, bedankt voor het "paranimfen" en Auke, mooie omslag!

Pap, mam, Marijke en Thijs, net als altijd kreeg ik ook de afgelopen jaren al jullie vrolijkheid, advies en steun, jullie zijn geweldig en echt ontzettend bedankt voor alles.

Marta e Afonso, a alegria de fazer parte da vossa vida é indescritível. Obrigado por tudo. Amo-vos.

Curriculum Vitae

Gert-Jan van der Heijden was born in Rotterdam on the 7th of July 1980. He graduated from high school (Huygens Lyceum, Voorburg) in 1998. After a year work and travel in Australia his medical training started in Amsterdam at the Vrije Universiteit. The scientific research segment of his medical curriculum took place in Pietersburg, South Africa and focussed on the metabolic syndrome in rural villages around Pietersburg.

Medical School graduation (2006) was followed by work as a resident at the Department of Pediatrics of het Medisch Spectrum Twente. During this time Prof. dr. P.J.J. Sauer (Beatrix Kinderziekenhuis, UMCG, Groningen) brought him in contact with Prof. dr. A.L. Sunehag (Children's Nutrition Research Center, Houston, United States). She gave him the opportunity to move to Houston, Texas, to join her research projects. The work on these investigations resulted in this thesis. During the two year stay at the CNRC in Houston he was granted a position in the Postdoctoral Research Training Program. Subsequently he received a position as a scientific investigator at the UMCG in Groningen to finish his dissertation. In January 2010 he started his training in Pediatrics at the Beatrix Kinderziekenhuis, UMCG, Groningen (head: Prof. dr. H.J. Verkade). He is together with Marta Vasconcelos and Afonso.